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African Journal of Food Science

Full Length Research Paper

Risk factors associated with retail meat vendors in Lubumbashi, Democratic Republic of Congo

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Food safety risk factors associated with retail meat sales in Lubumbashi, Congo D.R., was assessed in 2013. The study involved 168 meat vendors. The methods used were an interview and direct observation. Females represented 55.9%, and males represented 44% of the study respondents. Their age ranged from 18 to 40 years (92.8%). Sixty percent had obtained a primary level of education, 38% a secondary level, and only 1.1% completed the university level of education. Sixty-nine percent had fixed activities, and 30.9% were mobiles. None had a training certificate in food safety or hygiene practices. Overall, the poor level of knowledge/attitude and practices was associated with low level of education and the age of the vendors (<40 years) ($p<0.05$). Gender did not play a role ($p>0.05$). Attitude, practices, and lack of food safety knowledge in meat handling, improper slaughtering processes, poor environmental and personal hygiene, inadequate storage of food and lack of potable water were identified as major risk factors which may contribute to various contamination of meat sold at retail outlets in Lubumbashi.

Key words: Retail meat vendors, attitudes, practices, knowledge, food safety, Lubumbashi, Congo D.R.

INTRODUCTION

Foodborne diseases are a significant cause of morbidity and mortality worldwide. The World Health Organization (WHO) estimated in 2010, 600 million foodborne illnesses and 420 million deaths worldwide; 91 million persons are affected in the developing world (WHO, 2015). In USA, the FoodNet identified 25.606 infections, 5.893 hospitalizations, and 120 deaths during 2018 (CDC, 2013). In developing countries, around 2.2 million of deaths are due to foodborne illnesses (Imathiu, 2017). There are up to 2.5 million deaths of children due to diarrheal disease, according to WHO (WHO, 2008). Besides, the foodborne diseases' prevalence could be much higher in developing world due to poor hygiene conditions, lack of running potable water, ignorance of

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basic food safety notions, improper handling of food and ignorance of storage processes of foodstuff (Stratev et al., 2017). Considering the regional difference, Africa shows the highest burden per population of foodborne diseases (Iwu et al., 2017). Mishandling of foodstuff may lead to spread of the initial contamination of raw food and cross-contamination of cooked food with pathogens such as bacteria and lead to foodborne diseases (Akabanda et al., 2017). Food handlers play a crucial role in ensuring food safety throughout the chain of production, processing, storage and preparation (Mathenge et al., 2017).

Many studies in developing countries have reported that food handlers were implicated as an important factor of foodborne outbreaks. They are usually uneducated, and they have shown poor knowledge of adequate hygienic practices during food preparation, processing and storage (Choudhury et al., 2011; Stratev et al.; 2017; Alamo-Tonelado et al., 2018). Indeed, food handlers who had knowledge of good practices of safety while handling food could help to control foodborne illnesses as they are in direct contact with food especially cooked or ready to eat foods (Lee et al., 2017). Foodborne diseases associated with consumption of contaminated food with pathogens like *Campylobacter*, *Salmonella, Escherichia coli* O157:H7 and virus have been reported (Weam et al., 2016, Osimani et al., 2017; Shafini et al., 2017). Food products such as meats and meats products may harbour hazards like pathogens and their toxins or various contaminants or chemical residues; thus, they are considered as high-risk commodities (Haileselassie et al., 2013).

Consumption of meat and meat products sold at retail outlets has increased in the past years in Lubumbashi, DRC. Like in many developing countries, this phenomenon has economic implications. Since, this activity creates self-employment and provides meat/food at an affordable price to a large community of local population (Lamin-Boima, 2017; Ma et al., 2019). Around 2.9% (480.000 persons) of the population of Dhaka in Bangladesh depend on the income generated by street food vendors (Khairuzzamann et al., 2014). In Zambia, in 2003, street food sales employed around 16.000 people and had a turnover of 100 million US dollars (Imathiu, 2017). However, in most developing countries, street food vending activities are not under the regulation and protection of the government (Minh, 2017). In general, street food vendors operate in unsuitable hygienic conditions with lack of primary facilities. And there is evidence that street food may be exposed either to spoilage or pathogenic micro-organisms (Redzwan, 2016; Ekka, 2017).

Little is known about vendor's knowledge, attitudes, and practices in food safety of meat sold at retail outlets in the open market and along roadsides in Lubumbashi. The hygienic conditions in which meats are sold may lead to a significant threat to public health. In open markets, in Lubumbashi meats are usually exposed at ambient temperature, on inappropriate structures, or directly on the floor. Also, there was a lack of potable water, storage facilities such as refrigeration, garbage disposal and primary facilities such as toilets. Shortage of data on the quality of retail meat sold in Lubumbashi and on meat handlers' behaviour as well as the risk factors related, has increased our concern.

The aim of this study was (i) to assess the food safety knowledge, attitudes and practices among retail meat vendors in Lubumbashi and; (ii) to report the risk factors in common places of meat distribution.

Information gathered in this study could be useful for the local government for evaluation of food safety policies to improve good hygiene practices in street food and meat handling in Lubumbashi.

MATERIALS AND METHODS

Data collection

Data were collected in 2013 from 6 municipalities of Lubumbashi city (Lubumbashi, Kenya, Kamalondo, Katuba, Kampemba and Rwashi). The main places of raw meat and ready-to-eat (RTE) meat selling sites were investigated. They were constituted of 12 open markets and 49 snack bars.

The study involved 168 meat vendors, of which 116 operated in open markets and 52 in snack bars. In markets, the first group of 104 respondents was vendors of fresh meat, among them 90 females and 14 males. The second group of 12 respondents was vendors of smoked game meat, among them 4 females and 8 males. However, all respondents in snack bars were males.

Methods

Two methods were used: Interview and direct observation. The interview was based on structured and straightforward questions translated from French into local languages (Swahili and Lingala) if needed. The expected answer to the questions was qualitative: YES or NO. Sometimes a classification was requested. The collected information included:

Socio-demographic characteristics such as gender, age, marital status, level of education and type of activity (whether the vendor had a mobile or a fixed activity).

Knowledge, attitudes and practices in meat/food handling were assessed by answering our questions. Each response was given a score "1" for a "YES" response or a score "0" for a "NO" response. The total of scores was counted and converted to a percentage based on the total number of characteristics. We assessed the level of knowledge/attitude or practices as poor for the rate < 50%; as fair, for between 50-69%; and as good for 70% and more.

In case of doubt, a classification from 1 to 5 was requested, and additional explanations from vendors were accepted. A "YES" response was considered for a ranking from 3 to 5 and given a score "1". For a ranking of 1 to 2 the response was regarded as "NO" and given a score "0".

The direct observation concerned the characteristics and hygiene of places where meat was sold, the behaviour of vendors, their personal hygiene, and their practices while handling meat or other

Table 1. Socio-demographics characteristics of meat vendors in Lubumbashi.

Characteristics	Frequencies (n = 168)	Percentages
Gender		
Female	94	56.0
Male	74	44.0
Age (years)		
18 - 40	156	92.9
> 40	12	7.1
Marital status		
Married	94	56.0
Single	74	44.0
Education		
Primary	102	60.7
Secondary	64	38.1
High level	2	1.2
Vending places		
Market	116	69.0
Snack bar	52	31.0

products.

Statistical analysis was carried out with SPSS 23, for multivariable regression analysis. Chi-square was used to assess the association between variables and the p-value was considered significant at the level of 0.05.

RESULTS

Socio-demographic profiles of meat vendors in Lubumbashi

The socio-demographic characteristics of meat vendors are recorded in Table 1. There was a difference in gender; women represented 55.9%, and men represented 44%. This difference was very significant in markets (p=0.001), where 81% of vendors were women, and only 18.9% were men. In some cases, women were accompanied by their babies or pre-school age children. In snack bars, 100% of vendors were male as the goat slaughter process, and cooking of RTE goat skewers are handled only by men.

The average age was of 18 to 40 years (92.9%). Most of the vendors were married (56%). Many vendors (60.7%) had obtained primary level of education, which was the lowest. Thirty-eight percent (38%) had completed secondary level of education, and only 1.1% had a university level of education.

Among all the vendors, 69% had a stationary activity, while 30.9% of them were mobile. The mobility was concerning workers in snack bars, particularly because they were employed in many other snacks bars of the city.

None of the vendors in Lubumbashi was qualified to handle meat, and no one had a training certificate in food safety or hygiene practices.

Knowledge, attitude and practices of meat vendors

There was a poor knowledge, attitude and practices toward basic hygiene rules such as hand washing and body hygiene among meat vendors in Lubumbashi (Tables 2 and 3).

Only 32.7% of meat vendors washed their hands after using toilet facilities, 13.6% after the goat slaughter process and 0% after handling other products such as money and live animals. Only 39% of vendors used clean water to clean their vending sites and, 53.3% their utensils. Besides, 29.7 and 47% used wastewater to clean their vending places and utensils, respectively. Some vendors (19.6%) performed a dry cleaning of their vending places with brush; others (26.1%) used cloth to wipe their utensils at the end of daily activities.

Concerning personal hygiene, 61.3% of vendors were aware of taking a shower before they leave home to go for their activities, and 88.7% wore clean clothes. Thirty-

Table 2. Hygiene practice on sell places.

Parameter	Frequences of answer (%)				
	Markets (n=116)		Snack bars (n=52)		Total (n=168)
	Yes	No	Yes	No	Total of yes
Cleaning the place of work					
Mandatory day once a week (in markets)	116 (100)	0	-	-	116 (69.0)
Everyday (Snack bars)	-	-	52 (100)	-	52 (30,9)
Personal initiative (Markets and snack bars)	65 (56.0)	51 (43.9)	-	-	65 (38.6)
I pay someone to do it for me*	43 (37.0)	73 (62.9)	17 (32.6)	35 (67.3)	60 (35.7)
Before starting activities	116 (100)	0	52 (100)	0	168 (100)
After the activities	0	116 (100)	0	52 (100)	0 (0)
Cleaning was achieved					
With clean water	46 (39.6)	70 (60.3)	21 (40.3)	31 (59.6)	67 (39.8)
With waste water	27 (23.2)	87 (76.7)	23 (44.2)	29 (55.7)	50 (29.7)
Dry clean with a brush	28 (24.1)	88 (75.8)	5 (9.6)	47 (90.3)	33 (19.6)
Cleaning utensils					
After each usage	29 (17.2)	87 (75.0)	0	0	29 (17.2)
At the end of the day	76 (65.5)	40 (34.4)	45 (86.5)	7 (13.4)	121 (72.0)
When handling different products	21 (42.5)	95 (81.3)	0	52 (100)	21 (12.5)
Cleaning utensils with					
Clean water	70 (60.3)	46 (39.6)	23 (44.2)	29 (55.7)	93 (55.3)
Waste water	44 (37.9)	72 (62.0)	35 (44.3)	17 (32.6)	79 (47.0)
With a dry cloth	38 (32.7)	78 (67.2)	6 (11.5)	46 (88.4)	44 (26.1)

Those who delegate the cleaning process don't know how it was carried out. -, Not applicable.

Table 3. Knowledge of personal hygiene among meat vendors in Lubumbashi.

Parameter	Frequency of answers « yes » (%) All meat vendors (n=168)
Body hygiene before work	103 (61.3)
Wearing clean clothes for work	149 (88.6)
Wearing hair protection and gloves while selling	0
Wearing jewels and watch	67 (39.8)
Carrying a mobile phone	125 (74.4)
Washing hand (Without soap)	
After tip to toilet [a]	55 (32.7)
After touching money and other products than meat	0
After changing baby nappy	0
After contact with live animals	0
After the slaughter process	23 (13.6)
After cleaning stand and cooking stall	13 (7.7)
Temporary cessation of activities	
In the case of diarrhoea	41 (24.4)
In the case of Typhoid fever [b]	51 (30.3)
Other diseases	20 (11.9)
Voluntary detection of infections[c]	26 (15.4)

[a]If water is available. [b]According to the vendor's perception. [c]Excessed by the multiple requests for voluntary screening for infections.

Table 4. Global scores of knowledge and attitude among meat vendors.

Variable	Frequencies (n = 168)	Percentages (%)
Body hygiene	103	61.3
Wearing Clean clothes	149	88.7
Wearing Hair and hand protection	0	0.0
Wearing Jewels and Watch	67	39.9
Carrying a Mobile phone	125	74.4
Stop the activity in case of diarrhoea	42	25.0
Stop the activity in case of typhoid fever	41	24.4
Stop the activity in case of other diseases	16	9.5
Voluntary detection of diseases	19	11.3
Level of knowledge		
< 50% (Poor)	141	83.9
50 - 69% (Fair)	22	13.1
70%+ (Good)	5	3.0

Table 5. Global scores of hygiene practices among vendors

Variable	Frequencies (n = 168)	Percentages (%)
Hand washing after toilet	55	32.7
Hand washing after slaughter	23	13.7
Hand washing after the cleaning process	13	7.7
Cleaning vending places 1 mandatory day	116	69.0
Cleaning every day	52	31.0
personal initiative	65	38.7
With clean water	67	39.9
With wastewater	51	30.4
Dry cleaning	33	19.6
Cleaning utensils with clean water	93	55.4
Cleaning utensils with wastewater	78	46.4
Cleaning with a cloth	44	26.2
Level of practice		
< 50% (Poor)	139	82.7
50 - 69% (Fair)	29	17.3
70%+ (Good)	0	0.0

nine per cent (39.9%) were wearing hand jewels and watch, and 74.4% were carrying their mobile phones.

In the case of diseases, 24.4 and 30.4% were willing to stop their activities if they suffer respectively from diarrhoea or typhoid fever. None had a health certificate, and only 15.4% were interested in a voluntary screening of diseases.

Regarding the global score, only 3% of vendors had a good level of knowledge/attitude toward food hygiene practices. Thirty per cent (13.1%) had a fair level, and 83.9% had a poor level (Table 4). The poor level was significantly associated with the age of vendors (18-40 years) (p=0.004), the primary level of education (p=0.013), and their mobile activity (p= 0.046). The gender did not play a role (p=0.585) (Table 6).

None of the vendors had obtained a score of good practices toward food hygiene safety. However, 17.3% had a fair level of practices, and 82.7% had a poor level (Table 5). In this case, also, the poor level was associated with the age of vendors (18-40 years) (p=0.000). But vendors who had obtained a secondary level of education showed the poorest level of practices

Table 6. Association of vendors' characteristics with the level of knowledge of food hygiene practices.

Characteristics	Level of knowledge			Total (%)	Test* and p-value
	< 50% (Poor)	50 - 69% (Fair)	70%+ (Good)		
Gender					
Female	78(83)	14(14.9)	2(2.1)	94(100)	χ^2= 1.07; df = 2; p = 0.585
Male	63(85.1)	8(10.8)	3(4.1)	74(100)	
Age					
18 - 42 years	135(86.5)	16(10.3)	5(3.2)	156(100)	χ^2= 11,05; df = 2; p = 0.004
> 42 years	6(50)	6(50)	0(0)	12(100)	
Education					
Primary	91(89.2)	8(7.8)	3(2.9)	102(100)	
Secondary	50(78.1)	12(18.8)	2(3.1)	64(100)	χ^2= 12.62; df = 4; p = 0.013
High level	0(0)	2(100)	0(0)	2(100)	
Type of activity					
Fixe	93(80.2)	18(15.5)	5(4.3)	116(100)	χ^2= 6.18; df = 2; p = 0.046
Mobile	48(92.3)	4(7.7)	0(0)	52(100)	

Table 7. Association of vendors' characteristics with the level of food hygiene practices.

Characteristics	Level of practice		Total (%)	Test and p-value
	< 50% (Poor)	50 - 69% (Fair)		
Gender				
Female	78(83)	16(17)	94(100)	$\chi^2_{(a)}$= 0.01; df = 1; p = 0.926
Male	61(82.4)	13(17.6)	74(100)	
Age				
18 - 40 years	132(84.6)	24(15.4)	156(100)	$\chi^2_{(b)}$; p = 0.000
> 40 years	7(58.3)	5(41.7)	12(100)	
Education				
Primary	80(78.4)	22(21.6)	102(100)	
Secondary	59(92.2)	5(7.8)	64(100)	$\chi^2_{(c)}$= 13.11; df = 2; p = 0.001
High level	0(0)	2(100)	2(100)	
Type of activity				
Fixe	96(82.8)	20(17.2)	116(100)	$\chi^2_{(c)}$= 0; df = 1; p = 0.992
Mobile	43(82.7)	9(17.3)	52(100)	

(a) Chi-Square of Pearson Test; (b) Fisher's exact Test ; (c) Likelihood ratio.

(p=0.001) than their colleagues less educated. Gender and type of activity did not reveal any difference in practices (p=0.926 and p=0.992, respectively) (Table 7).

Risk factors associated with retail meats vendors

In this study, we identified many risk factors associated with the environment where the meat was sold and with the vendors' practices during their activities. Among them are as follows:

The conditions of meat vending

In the market, meats were directly displayed on concrete

stalls or wooden table, or directly on the floor. Plastic, cardboard, old newspaper or other materials were used as a tablecloth. There were no scopes to avoid insect or dust during the display.

The meat processing and handling

Vendors used knives, metal saw, axe and machete to cut the meat into small pieces weighing 50 to 120 g. All the handling processes were achieved with bare hands. Sometimes, meats of different species, fish and vegetables were sold by a single vendor without a proper separation.

The absence of a refrigeration system, improper storage process and packaging

There was no refrigeration system during vending activities. Vendors kept unsold meat in deep freezer of market's warehouse. Thus, the meat was frequently frozen and unfrozen. In snack bar also, raw meat and cooked skewers were kept at ambient temperature.

In the market, clean and unused polythene bags were used for packaging. However, in snack bars, RTE goat skewers were served on plastic or metallic plates to consumers who eat in the snack bar. For those who took away their dishes, old papers or newspapers, as well as reusable polythene bags, were used for packaging.

The slaughtering processes

There is no formal abattoir in Lubumbashi dedicated to goat slaughter. In general, goats were slaughtered in a backyard, in the market or along roadsides. The slaughter process was carried out with the bare hands. Those who achieved this process did not wear aprons, hair protection or rubber boots. Most of them wore regularly dirty clothes as work clothes to do their duties.

The use of ingredients

Fresh onions, white vinegar, salt, cooking oil and in some cases nitrites salts were used for seasoning of skewers. Besides, meat handlers used secret seasoning, which was reported to be the key to success and made the vendors famous in this field.

The access to water and absence of waste management system

Vendors had no permanent access to clean and potable water. Most of the time, water was purchased, in polythene bags, from street vendors of water. However, in the rainy season, vendors use rainwater. In all cases, water was stored in a single bucket and used for washing hands, stands and utensils.

Both in market and snack bars, there was no defined waste management system. The waste was disposed on the floor at the vending site, and often in an illegal unloads around the market. Moreover, after goat slaughter, usually the gastro-intestinal contents, wastewater, and all the garbage were disposed of in the public sewers.

DISCUSSION

This study was conducted to assess the knowledge, attitude, and practices among meats vendors in Lubumbashi, and to identify risk factors associated with retail meat vendors in Lubumbashi.

Socio-demographic characteristics

Most of the vendors were women. The difference in gender was significant in the market where 81% were women (p=0.001), and 18.9% were men. Similar results were reported in many other studies, where women were also much involved in food street vending activities compared to men (Martins, 2006; Lee et al., 2017). However, in snack bars in Lubumbashi, all the vendors were men as the slaughter of goat is carried out only by men. A similar finding was reported in Nairobi. Indeed, the survey was conducted in establishments dealing with animal products such as butcheries where most of the workers were males (Mathenge et al., 2017). In countries such as Pakistan, Malaysia and Taiwan, men were also the most representatives (Zeeshan et al., 2017).

The average age of the respondent to our survey was 18 to 40 years (92.9%). The same range of age of street food vendors and food handlers was reported in other studies (Meleko et al., 2015, Afolaranmi et al., 2015). In Lubumbashi, most of the vendors were married (56%). Like finding in Nairobi in Kenya, where 55.7% of street vendors were also married.

The majority (60.7%) of meat vendors in our survey had obtained a primary level of education, which was the lowest. Thirty-eight (38%) had achieved a secondary level of education, and only 1.1% had a university level of education. In Haiti, 22.5% of street vendors were illiterate; 45% had a primary level of education, 26.2% a High school level and 6.3% a university level (Sapamundo et al., 2015).

Most of the vendors in Lubumbashi had a stationary activity (69%), while 30.9% of them were mobile. Similar results were reported in Sudan, where 64 % of the vendors had fixed activities (Abdalla et al., 2009). Contrary to our study, in Benin, 76.7% of the street food vendors surveyed were mobile (Ohin et al., 2018).

FAO and WHO recommend that food vendors must hold a basic training in food safety and hygiene practices by relevant authorities before they are licensed for the activity. Still, in Lubumbashi, none of the vendors was qualified for handling meat, and no one had a training certificate in food safety or hygiene practices. Studies suggested that vendors trained in food safety hygiene had a good level of knowledge and practices of hygiene when handling food (Rahman et al., 2012; Adane et al., 2018). Despite this, in China, there was no significant difference in food safety knowledge among vendors who had received training in food safety and those who did not train (Ma et al., 2019).

Knowledge, attitude and practices

In our study, only 3% of meats vendors had a good level of knowledge/attitude toward food hygiene practices while the majority had a poor score (81.9%), and 13.1% had a fair score. Regarding their practices in hygiene and food handling, none of the vendors had a good score. The majority had a poor score (82.7%), and the others had a fair score (17.3%). In Owerri Town in Nigeria also, vendors who had a poor level of knowledge in food hygiene showed poor hygienic practices (Iwu et al., 2017). Similar findings were reported in developing countries like Soudan and Western Africa, among others (Barro et al., 2007; Abdalla et al., 2008).

Age and gender have been discussed in the knowledge, attitude and practice of hygiene in the handling of meat and food products. In our study, vendors aged under 40 years and less educated, appeared to be associated with a poor level of knowledge/attitude ($p=0.004$). Besides, the same group of vendors (< 40 years) showed a poor level of food hygiene practices ($p=0.000$), although they had obtained a secondary level of education. But in both cases, there was no difference between genders. In a study conducted in Ghana, there was no association between the vendors' ages with the knowledge and practice of food hygiene (Dun-Dery and Addo, 2016). But another study in the same country showed that the group of vendors aged from 36-45 years has a high awareness of food hygiene (Dajaan et al., 2018). Like in our study, in Uganda also, the low level of education of vendors was associated with poor knowledge of hygiene practices during food handling and preparation (Muyanja et al., 2011). In Nepal, for instance, food sold by illiterate vendors showed a higher incidence of bacterial contamination (Khadka et al., 2018). In USA, there was an association between gender, age and level of education. Indeed, women appeared to provide the best hygienic quality of food compared to men. However, these women were at least 40 years old and had an instruction-level of secondary school (Omemu and Aderoju, 2008).

Although hands are recognized to be a significant vehicle of biological hazards; in this study, only 32.7% of meat vendors washed their hands after using toilet facilities. This percentage was of 13.6% after the goat slaughter process. But all vendors never wash their hands after handling other products such as money and live animals. In India, most of the vendors did not wash their hands, and they handled money with bare hands while serving food (Reddi et al., 2015). In Brazil, 67% of street food vendors were reported to wash their hands at least 4 times a day while working. Besides, 33% of them did not wash at all their hands, and others used only water without detergent (Cortese et al., 2016). For hand washing to be considered as effective, it must be carried out with a detergent such as soap and hands must be well rinsed and wiped preferably with disposable papers (Bloomfield and Scott, 1997; CDC, 2013, Galgamuwa et al., 2016).

Concerning body hygiene, 61.3% of meat vendors in Lubumbashi were aware of taking a shower before and after the day of work. Most of the respondents (88.6%), of which the majority were women, considered it useful to wear clean clothes for work, but there was no association between gender and the level of knowledge/attitude and practices in food safety and hygiene practices ($p>0.05$). This corroborated with the findings of Malavi et al. (2017).

In our study, none of the vendors was wearing hair protection or gloves. But, most of them wore hands jewels and watches (39.8%), and 74.4% were frequently using their mobile phones. Vendors in India did not wear hair protection, and 28% were scratching their scalp while preparing food. In addition, 60% had long fingers nails, and 64% wore jewelry (Reddi et al., 2015). Only 20.8% of vendors covered their hair in Ethiopia (Amare et al., 2019). Although in Nigeria, vendors were aware of keeping their bodies and clothes clean, they did not consider as necessities to wear hair protection, to remove hand and arm jewelry and to keep nails short (Omemu and Adejoru, 2008). The poor personal hygiene was pointed out in contamination of street food. Indeed, lack of body hygiene and wearing hand jewelry or other accessories were established as a high-risk factor of cross-contamination between food and food handler's hands, cuts or sores, mouth, skin, and hair (Campos et al., 2009; Rane, 2011). Thus, good personal hygiene must be considered for meat handlers to avoid cross-contamination from them to the meat during handling, vending or storage processes.

In Lubumbashi, meat vendors had not a health certificate which allows them to practice activity in direct contact with fresh or cooked meat. Besides, only 15.4% were interested in screening for infection voluntarily. Though, medical examination of food handler is essential for the control of transmissible diseases from food handlers to the food they are manipulating. Similar results

were reported in Dhaka in Bangladesh, where most of the vendors had not a health certificate or a license to operate in their business (Khairuzzamann et al., 2014). Regulatory authorities in Ghana recommend that foods vendors must be screened for transmissible diseases at least every six months. However, during an investigation, 71% of vendors surveyed were tested, and 64% of them had the screening more than six months ago (Apanga et al., 2014).

In our survey, vendors were aware of foodborne diseases such as cholera and typhoid fever. But they did not apply basics hygiene rules like washing hands with clean water when handling food. In most developing countries, street food vendors were not completely ignorant of food safety practices, but they could not practice them (Baluka et al., 2015; Galgamuwa et al., 2016; Ohin et al., 2018). Nevertheless, 24.4 and 30.4% of meat vendors in Lubumbashi were willing to stop handling meat if they suffer from diseases such as diarrhoea and typhoid fever respectively. In Nigeria, also, in the case of diarrhoea, 24% of street food vendors were reported willing to stop their activities (Omemu and Aderoju, 2008). During our study, some of the meat vendors reported that they suffered, at least once, of diarrhoea or typhoid fever in the last 3 months before our survey. However, none had to stop his activity because they could use auto medication or use traditional remedies to get rid of the disease. In Bloemfontein, South Africa, street food vendors prepared food for public consumption while sick, and they could not stop their activity because their living depends on the daily return of the trade (Alami, 2016). In Ethiopia, food handler has been reported to suffer from diseases like diarrhoea, cholera, typhoid fever (Tesfaye, 2018). Studies showed that pathogens were frequently isolated in contaminated street food (Shaltot et al., 2015; Amare et al., 2019). Thus, foods handlers may lead to cross-contamination of food with bacteria when they suffer from specifics diseases.

In Lubumbashi, 39% of meat vendors claimed to use clean water to clean their stands and 55.3% their utensils. Otherwise, 29.7% of respondents used wastewater to clean their stands and 47% to clean their tools. Similar results were reported in Jigjiga in Ethiopia, where 39.4% of vendors washed their utensils with dirty water which was used several times (Bereda et al., 2016). In this study, dry cleaning was performed by 19.6 % of respondents using a brush for cleaning their stands, and 26.1% used a cloth to wipe their utensils at the end of daily activities. These practices were reported before among street food vendors in many studies (Barro et al., 2007; Ologhobo et al., 2010). Utensils were frequently reported to be the vehicle of cross-contamination of street vended foods. The contamination of the raw products and recontamination of RTE products result in most cases from the use of utensils that were not thoroughly cleaned; or when good hygiene practices were not applied by food handlers (Taulo et al., 2008; Carrasco et al., 2012). Furthermore, using a cloth to wipe dirty utensils can be considered as a risk factor of contamination of food and other surfaces. Gorman et al. (2002) found dishcloths to be contaminated with pathogens following chicken preparation in a domestic kitchen in Ireland. Thus, these pathogens may be transferred onto many other surfaces and food during the handling process.

Risk factors

During our survey, we identified many risk factors which may contribute to food contamination. Regarding vending sites, in Lubumbashi, meats were vended in poor sanitary conditions. It was directly displayed on concrete stalls, wooden tables, plastic or other materials. The meat was exposed to insect and dust. In Jigjiga also, vending places were in the wooden stall, canopies and polythene containers (Bereda et al., 2016). Wooden stalls and other surfaces may act as risk factors since they were associated with contamination of meat. Indeed, microorganism's niches and environmental pollution were found on those types of surfaces. For instance, bacteria were recovered from a wide range of food contact surfaces, including stainless steel, rubber, wood, polystyrene and glass material (Mafu et al., 2011; Patrignani et al., 2016).

Poor vending conditions were also reported in many studies. Indeed, vendors handled foods in unsanitary conditions at ground level and had their food exposed to flies, rats and cockroaches (Ehiri et al., 2001; Alamo-Tonelado et al., 2018). The presence of dust and flies are risk factors as they may harbour pathogens and lead to contamination of food. Indeed, flies and the environmental conditions such as water, dust, rains, winds, and urbanization were identified as vectors of zoonoses and contamination of street vended food (Adeyemo et al., 2009).

Concerning meat processing and handling in Lubumbashi, the meat was cut into small pieces to be sold at affordable prices. Still, all the handling processes were achieved with bare hands. Moreover, there was no proper separation between meats of different species, fish and vegetables. Food of animal origins such as meat and fish and their products are considered as high-risk commodities regarding pathogens, natural toxins and other contaminants they may harbour (Novoslavskij et al., 2016; Boukili et al., 2019).

Furthermore, meat processing was identified as a significant risk of meat contamination. Indeed, cutting, mincing, handling or packaging process may lead to cross-contamination between material or allow bacteria presents on the surface to spread and distribute homogeneously in the meat (Abongo'o and Momba, 2009;

Shilenge et al., 2016; Shafini et al., 2017). In Nairobi, sausages had the highest count of coliform due to post handling process, which involved the cutting of the sausage (Kariuki et al., 2017). Moreover, mixing different type of food may originate in the cross-contamination of safe products (Cadirci et al., 2010).

In our survey, there was no refrigeration system both in snacks bars and markets. Besides, we noticed inadequate storage processes and packaging. Unsold cooked skewers were kept at ambient temperature and reheat to be sold the following day. The unsold meat was held in a deep freezer in the market's warehouses and unfroze the next morning. Keeping the perishable food such as meat in an inadequate range of temperature or refreezing a completely thawed food is considered as risk factors since these processes may accelerate the growth of contaminating bacteria (Akabanda et al., 2017; Mercier et al., 2017). Like in Lubumbashi, studies reported that vendors kept food at ambient temperature during vending activities (Cortese et al., 2016; Tesfaye, 2018). Vendors who kept leftovers food more than 2 days provided food of poor microbiological quality (Derbew et al., 2013). The storage of the food at an inadequate temperature can act as an incubator media for pathogens, whether the food is raw or cooked. Food which was held at an ambient temperature between 15-45°C for more than 4 h presented a significantly higher risk for public health (Tafesse et al., 2014).

In Lubumbashi's markets, vendors used clean and unused polythene bags for packaging. However, in snack bars, old papers, newspapers, cardboard and other inappropriate material were used for skewers packaging. These practices increase the risk of cross-contamination of food with various contaminants such as pathogens or chemicals. In Pakistan also, vendors served food in paper bags plain or with newsprint. These bags were made using glue which was not recommended by any food control authority (Ahmed et al., 2017). Packaging may be contaminated with spoilage or pathogenic microorganisms (Patrignani et al*.,* 2016). Indeed, bacteria and fungi were isolated from paper-based packaging made with recyclable material (Hladíková et al., 2015). Thus, attention should be paid to the material used for packaging since it may act as a source of contamination of food.

In Lubumbashi, goat 'slaughtering processes took place in the market, in the backyards and along roadsides with the bare hands, without formal equipment. There was no formal abattoir for goats. Unlike in Ghana, there were formal slaughterhouses. However, some vendors did not use them because of the distance from the chop bar to the abattoir and the exorbitant fees charged for ante-mortem inspection of livestock (King et al., 2000).

Absence of formal abattoir and improper slaughtering processes constitute risk factors of meat contamination through the production chain. Further cross-contamination of meat may result in abattoir environment or following contact with meat handlers, and thus increasing the risk of food contamination (Rouger et al., 2017).

In our survey, vendors used fresh onions, white vinegar, salt, cooking oil and nitrites salts for the seasoning of the skewers. However, some of the ingredients were kept secret because they were the key to success, according to vendors. Spices used in food preparation may act as a vehicle for biological or chemical hazard through food products. Spices such as black pepper, paprika and others may be contaminated by biological hazards when not handled properly (Carrasco et al., 2012). Like in our study, in South Africa, 56% of mobile vendors used unlabeled and unpacked spices, and 37 % purchased them from the traditional daily or weekly markets and prepared them themselves at home. And yet, studies have demonstrated that homemade condiments were contaminated with *Bacillus cereus* (Alami, 2016). In Nigeria, to increase the palatability of foods, vendors used spices such as crayfish and soybean. Unfortunately, these ingredients were subject to frequent contamination from vendors' unwashed hands and material used for packaging such as leaves, old newspapers and reusable polythene bags (Ehiri et al., 2001).

Vendors had no permanent access to clean and potable water in this study. They purchased water in polythene bags sold by street vendors of water; or used rainwater and stored it in a single bucket to be used for washing hands, stands and utensils. The same practices were reported in Ghana (Dajaan et al., 2018). Many studies have suggested that the lack of clean water was enhancing the possibility of cross-contamination between hands, work surfaces, utensils, and foods (Vollaard et al., 2004; Khadka et al., 2018). Pathogens present in water may contaminate surfaces, utensils and further, foodstuff. Thus, the lack of potable water may act as a potential risk factor for food poisoning.

In this study, we noticed the lack of a waste management system which contributed to environmental pollution. Indeed, vendors threw their garbage on their vending places, or illegally around the market, along roadsides or in public sewers. In Gauteng, South Africa, also, 56% of vendors poured wastewater into the storm water drainage system (Martins, 2006). Similarly, in Pakistan, 80% of street food vendors threw their wastewater along roadsides. Others threw their leftover foods in their vending vicinity and contributed to creating an unsanitary environment (Ahmed et al., 2017).

Conclusion

It was found from this study that meat was vended in

poor sanitary conditions in Lubumbashi. The lack of knowledge, attitude and practice of hygiene in meat handling by retail meat vendors appears to be a real threat to public health. Meat vendors must be regarded as a critical control point in the chain of distribution of meat of good hygienic quality. Furthermore, we identified that poor environmental conditions at the vending site, the lack of personal hygiene, the improper handling and storage processes as well as the lack of potable water and the waste disposal are important risk factors which may lead to various contamination of meat sold at a retail outlet in Lubumbashi.

Recommendations

These data may be used as a basis for evaluation of the food safety policies which would be set up, in joint effort with the public health authority, to improve Good Hygiene Practices in street food and meat handling in Lubumbashi. However, street food vending activities will not be prohibited as long as it contributes to the informal economy in the city. Local government, as well as regulatory authorities, may support these activities by recognizing them as a part of the economy. However, they should provide regulation and control to ensure food safety of meat vended at a retail outlet, and thus reduce the risk toward public health.

We highly recommend that the local administration provides primary facilities such as adequate vending places where minimal services are provided like potable water, energy, tile-flooring outlets, coated walls and waste management. Each point of meat sale should be equipped with a basic hygiene appliance containing a refrigerator if possible, with transparent displays to facilitate the customer's choice. Storage warehouses in the market must be equipped with refrigerators or freezers to ensure better storage of meats.

We also recommend that the local authorities provide conventional goat's slaughterhouses and request minimal training in goat slaughtering for those who are involved in this practice.

Furthermore, the local government should raise awareness of the threat of improper practices in meat/food handling and set up control measures to oversee meat vending activities in markets and streets. For instance, vendors must be licensed before they practice their business; and they should undergo medical screening for transmissible diseases. Meat vendors should be aware of the role they play in the transmission of foodborne illnesses.

Finally, an educational program and food safety training should be considered for persons dealing with meat and street food. This program requires a substantial investment. But the cost/benefit of the educational program in good hygiene practice during meat/food handling is more economical than dealing with consequences of foodborne diseases due to lack of knowledge in food handling.

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CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Effect of process treatments on the proximate composition of tigernut-soy milk blends

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The effect of pasteurisation parameters and formulation components on the nutritional composition (carbohydrate, protein, fat, ash and moisture contents) of tigernut-soy milk blend was investigated. Twenty-six different formulations (F_1 – F_{26}) were prepared from tigernut and soy milk. The process treatments employed were; pasteurisation temperature, pasteurisation duration and mixing duration which varied from 60 – 80°C, 5-20 s and 5-15 min respectively. The results showed that F_{24} with blend constituents of 37, 5 and 50% of tigernut milk, soy milk, water and process treatments of 60°C, 20 s and 5 min of pt, pd and md respectively had the highest percentage of carbohydrate (12.53%) and fat (3.181%). The highest protein value (5.69%) was found in F_{11} with blend constituents of 27, 5 and 60% of tigernut milk, soy milk, water and process treatments of 80°C, 20 s and 5 min of pt, pd and md respectively. F_{23} with blend constituents of 11, 11 and 70% of tigernut milk, soy milk, water and process treatments of 80°C, 20 s and 5 min of pt, pd and md respectively had the highest ash (4.38%) content. The most abundant in moisture content (93.065%) was F_{18} with blend constituents of 5, 17 and 70% of tigernut milk, soy milk, water and process treatments of 60°C, 5 s and 5 min of pt, pd and md respectively. Pasteurised tigernut-soy milk is rich in nutritional quality.

Key words: Pasteurisation, mixing, temperature, milk, ash, nuts, moisture content.

INTRODUCTION

Tigernut (*Cyperus esculentus* L.) belongs to the division–*Magnoliophyta*, class–*Liliopsida*, order–*cyperales* and family–*Cyperaceae*. It is a cosmopolitan, perennial crop of the same genus as the papyrus plant (Belewu and Belewu, 2007; Adejuyitan, 2011). The tubers which are about the size of peanuts are abundantly produced in Nigeria. It has other names such as ground almond, *zulu* nut, *chufa*, yellow nutgrass, edible rush and rush nut. In Nigeria, Yorubas call it *Imumu*, Hausas *Aya*, the Igbos *Aki* Hausa; whereas in the Southern part of Nigeria it is known as *Ofio*. Since early times (chiefly in West Africa and South Europe), tigernut has been cultivated for its small tuberous rhizomes which are used as hog feed, eaten raw or roasted, or pressed for its juice to produce a beverage (Osagie and Eka, 1998).

The nuts have excellent nutritional qualities with fat composition similar to olive oil, and are also rich in mineral content especially phosphorus and potassium but with low sodium content (Martinez, 2003). According to Oladele and Aina (2007), the crude protein content of the

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Figure 1. Sample of the soya beans.

Figure 2. Sample of the Tigernuts.

nuts ranged between 7.15 and 9.7%. *Chuffa,* as it is also called is cultivated in Nigeria primarily because of its rich vegetable milk which is an alternative to cow milk among the rural poor. It is used in the production of yoghurt and *Kunnu* (beverage) to quench thirst in Northern Nigeria (Sowonola et al., 2005). Tigernut tubers have also been used as alternative to cassava in baking industry (Bosch et al., 2005).

Soybean belongs to the family leguminous, subfamily *papiliondase* and the genus *Glycine Max*. The total area cultivated when Soybean (*Glycine max*) was first introduced in Nigeria in 1908 was 401,000 ha, while the current yield is about 1270 kg per hectare (Rehman et al., 2007). Soyabean not only provides quality macronutrients but also various other micronutrients, which are otherwise required to fight against malnutrition. It is rich in protein content and can furnish protein supply to bridge up the protein deficiency gap at low-cost than any other crop (Rehman et al., 2007).

Milk has been recognized as an important food for infants and growing children. In developing countries, the cost of dairy milk and their products is prohibitive and this has led to the development of alternative source of milk from plant materials. Tigernut milk (having Spanish name *horchata*) is a refreshing purely natural vegetable drink and or dessert, which is prepared with water, sugar and tigernuts. It is a very nutritive, energy drink both for young and old. Soymilk not only provides protein but is also a source of carbohydrate, lipid, vitamins and minerals (Chien and Snyder, 1983). It is an alternate of dairy animal milk due to the fact that it is less expensive despite it having high-quality protein. It has also been proved to be a healthy drink and is important especially for people who are allergic to cow milk protein and lactose (Rehman et al., 2007).

Tigernut-soy milk is a blended, processed commodity and is a source of quality energy, protein, minerals, and vitamins; combining the nutritional benefits of both.

Modern pasteurization is the application of adequate heat to a product for a period of time for the purpose of destroying pathogenic microorganisms, yet leaving the product acceptable from sensory and nutritional stand point (Lewis and Heppell, 2000).

In developing countries, the cost of milk and its products is prohibitive. This has led to dramatic decrease in the consumption of milk and milk products and stimulated in part the processing of milk from different seeds and nuts (Belewu and Belewu, 2007). High price of imported milk and milk products coupled with poor milk production in Nigeria and Africa in general seem to have made consumers more readily accept milk produced from plant sources.

Therefore, this study sought to evaluate the nutritive value of soy milk and tigernut milk as well as their combination in various proportions as affected by pasteurization, with a view to incorporating this into local milk production in Nigeria, taking into cognizance the importance of heat treating the milk at different temperatures.

MATERIALS AND METHODS

The soya beans and tigernuts used for this research work were purchased from Kure Market, Minna, Niger State, Nigeria. The samples of soya beans and tigernuts used for this experiment are shown in Figures 1 and 2 respectively.

Procedure *for production of tigernut milk*

Samples of dried tigernuts were purchased from the market and the bad nuts and other foreign materials were picked out. The tigernuts were then washed and soaked in water (6 L: 1 kg) for 18 h. Soaking of the tigernuts in water helps to soften it so as to blend with ease. The method of Belewu and Abodunrin (2006) was used. The soaked tigernuts were milled into slurry which was then pressed using muslin cloth to extract the milk and the milk extracted was divided into three parts and diluted to 50, 60 and 70% with water respectively. The flow chart for the production of tigernut milk is shown in Figure 3. The filtrate was stored in a plastic container for further processing.

Procedure for production of soy milk

Five kilograms (5 kg) of soya beans was soaked for 18 h in 15 L of potable water to give a bean-water ratio of 1:3. The soaked beans

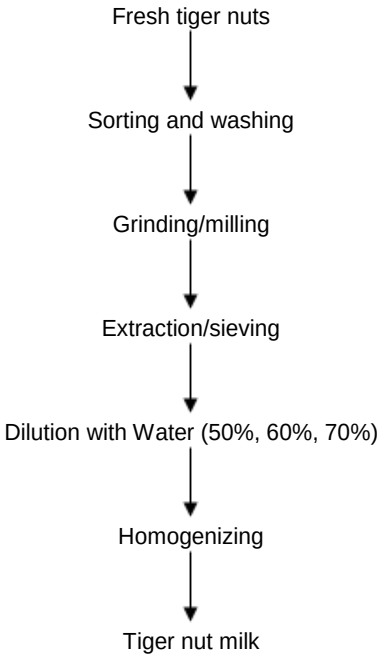

Figure 3. The flow chart for tigernut milk production.

was drained, rinsed with portable water and dehulled. Afterwards, the dehulled beans were milled. The resulting slurry was filtered through a muslin cloth and the extract (milk) obtained boiled for 2 h, after which it was divided into three parts and diluted with water to 50, 60 and 70% respectively. The flow chart for soymilk production is shown in Figure 4.

Procedure for preparation of tigernut-soy milk drink

Tigernut milk and soya milk were combined in varying proportions to obtain the final products as shown in Table 1. This was done using a LEXUS food blender operated at speed level one (450 rpm) for the duration specified for the various samples. The resulting blends were packaged in plastic bottles and were then pasteurized at the indicated temperatures and durations as specified for each of the samples in Table 1 using a water bath (Supplementary Figure 1 and 2).

After heating, they were cooled immediately to room temperature (28 ±2°C). The flow chart for tigernut-soy milk drink production is shown in Figure 5.

Design of the experiment

Mixture - process design was used for the experimental design. The design matrix (Table 1) generated twenty-six experimental runs (Table 2). The mixture variables are tigernut milk, soy milk and water. The process parameters include pasteurization temperature, pasteurization duration and (constituent) mixing duration. While the responses were carbohydrate, protein, fat, moisture and ash.

Proximate analysis

The proximate composition of samples (carbohydrate, protein, fat, ash and moisture contents) was determined according to the method described by the Association of Official Analytical Chemists (AOAC, 2000).

Statistical analysis

All experiments were carried out in triplicates. Data obtained were analyzed statistically using Design Expert 9.0 statistical package.

RESULTS AND DISCUSSION

The twenty-six separate food formulations and their corresponding results for the proximate analysis are presented in Table 3.

Effect of pasteurization and other process parameters on the proximate composition of tigernut-soy milk blends

Carbohydrate

The carbohydrate content of the samples ranged from 0.38 to 12.53% as shown in Table 3. From Figure 6, it can be observed that the carbohydrate value increased with increasing tigernut milk and decreasing soy milk in the blends when water was kept constant. Thus, the higher the percentage of tigernut milk in tigernut-soy milk, the more the carbohydrate content of the blend (Figure 7). More importantly, Figures 6 and 7 show that carbohydrate content of the various formulations generally reduced with increase in pasteurization temperature.

The analysis of variance (ANOVA) for the response surface combined linear × linear model of the carbohydrate yield is shown in Table 4. The model expression developed, that relates the carbohydrate yield and the six reaction parameters (A, B, C, D, E and F) is considered suitable because its p-value is less than 0.05. The model F-value of 5.14 implies that the model is significant. The F-value is the ratio of the Model SS/Residual SS and shows the relative contribution of the model variance to the residual variance. A large number indicates more of the variance being explained by the model. The model fit was also checked with the correlation factor R^2, which equals to 80.16%.

The significant factors from ANOVA analysis are the interactions between tigernut milk and pasteurization temprature (AD) with a p-value of 0.02 which is less than 0.05. Another significant factor is the interaction of tigernut milk and pasteurization duration with p-value of 0.03 (less than 0.05). The other factors of the model have no statistically significant effect.

In this study, the R^2 value of 0.8016 indicates that the variation of 80.16% is attributed to the independent variables while 19.84% of the total variations is not explained by the model. The value of the coefficient of

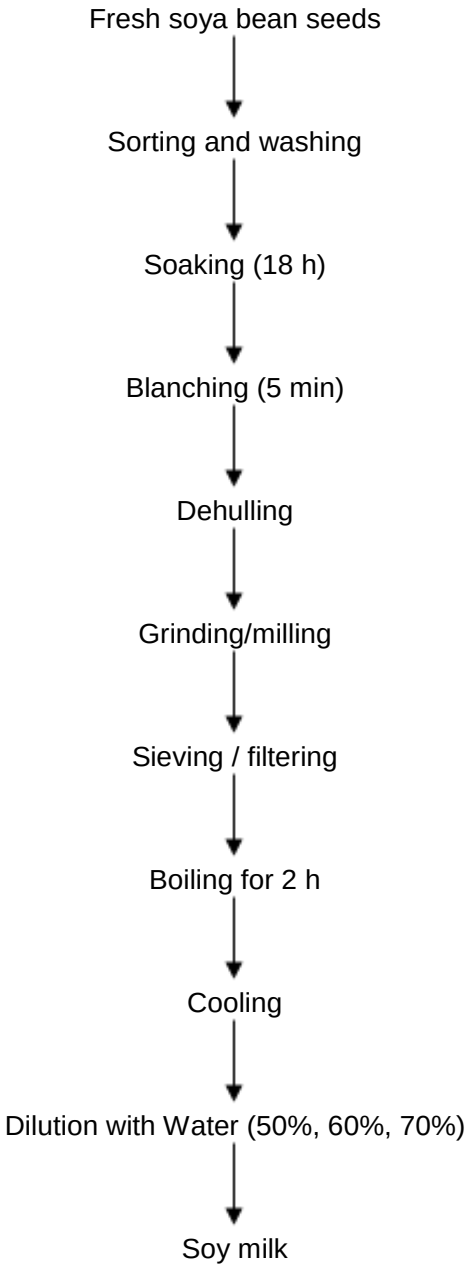

Figure 4. The flow chart for soymilk production.

variation CV%, 51.29 gives the precision and reliability of the experiment carried out where a lower value of CV indicates a better precision and reliability of the experiments carried out. Table 5 shows regression coefficients estimates of carbohydrate.

The regression analysis from Table 5 produced the following coded equation:

$$Y1: Carbohydrate\ Content = +6.45\mathrm{A} + 2.55\mathrm{B} - 1.33\mathrm{C} - 1.60\mathrm{AD} + 1.53\mathrm{AE} + 0.17\mathrm{AF} + 0.032\mathrm{BD} + 0.05\mathrm{BE} + 0.76\mathrm{BF} - (9.017\mathrm{E} - 003)\mathrm{CD} - 0.54\mathrm{CE} - 0.22\mathrm{CF} \quad (1)$$

The model equation (Equation 1) shows that all the positive coefficient terms such as A, B, AE, AF, BD, BE and BF indicate synergetic or favourable effect on the carbohydrate yield, while the negative coeffcients of the model terms such as C, AD, CD, CE and CF indicate antagonistic effect on the carbohydrate yield (Betiku et al., 2014).

The linear effect of A and B are the general determining factors of carbohydrate yield as they have the larger coefficients. The linear effect of C, interaction effect of AD and AE are secondary factors of the response. From this analysis, it can be seen that the tigernut milk concentration (factor A) has the highest coefficient among the three independent variables. This implies that the yield of carbohydrate from tigernut-soy milk blends relies greatly on this factor.

Protein

The protein content of the samples ranged from 3.28 to 5.89% (Table 3). There were fluctuations in the protein content of the tigernut-soy milk blends. The contour mix-process plot (Figure 8) gives a clear view of the protein yield in relations to the various process parameters, while the relationship between protein yield and the blend constituents is shown in Figure 9.

The fluctuation in the protein yield pattern may be attributed to the destructive effect of heat process involved in pasteurization on the amino acids as well as the tannin-protein complex which have contrast effects (Imafidon et al., 1997). Where amino acids are destroyed there is a consequent reduction in the total nitrogen which leads to decrease in the protein content of the resulting pasteurized milk (Oluwaniyi et al*.,* 2009), whereas, a rise in the amount of protein may be credited to the destruction of tannin-protein complex (Belewu and Belewu, 2007).

Generally, the range of protein content of the various formulations after pasteurization shows there are no serious adverse effect on the protein content as a result of pasteurizing the milk.

The analysis of the variance (ANOVA) for the response surface combined special cubic x mean model of the protein content of tigernut-soy milk bends is shown in Table 6. The model expression developed related the protein yield and the six reaction parameters considered (A, B, C, D, E, F); it has a p-value of 0.0526 greater than 0.05, which sugests there might be a more appropriate model for this experiment. The model F-value of 2.59 implies that the model is fairly significant. The model fit was also checked with the correlation factor R^2, which equals 44.99%.

The significant factors from the ANOVA analysis were: the interactions between tigernut milk and soy milk (AB) with a p-value of 0.0186 which is less than 0.05; the interaction of tigernut milk and water (AC) with p-value of

Table 1. Design matrix of the blend constituents and process treatments.

Name	Unit	Type	Low Actual	High Actual	Low Coded	High Coded
A - Tigernut Milk	%	Mixture	5.00	37.00	0.000	1.000
B - Soya Milk	%	Mixture	5.00	37.00	0.000	1.000
C - Water	%	Mixture	50.00	70.00	0.000	0.625
D - P/ Temperature	°C	Numeric	60.00	80.00	-1.000	1.000
E - P/ Duration	s	Numeric	5.00	20.00	-1.000	1.000
F - M/Duration	min	Numeric	5.00	15.00	-1.000	1.000

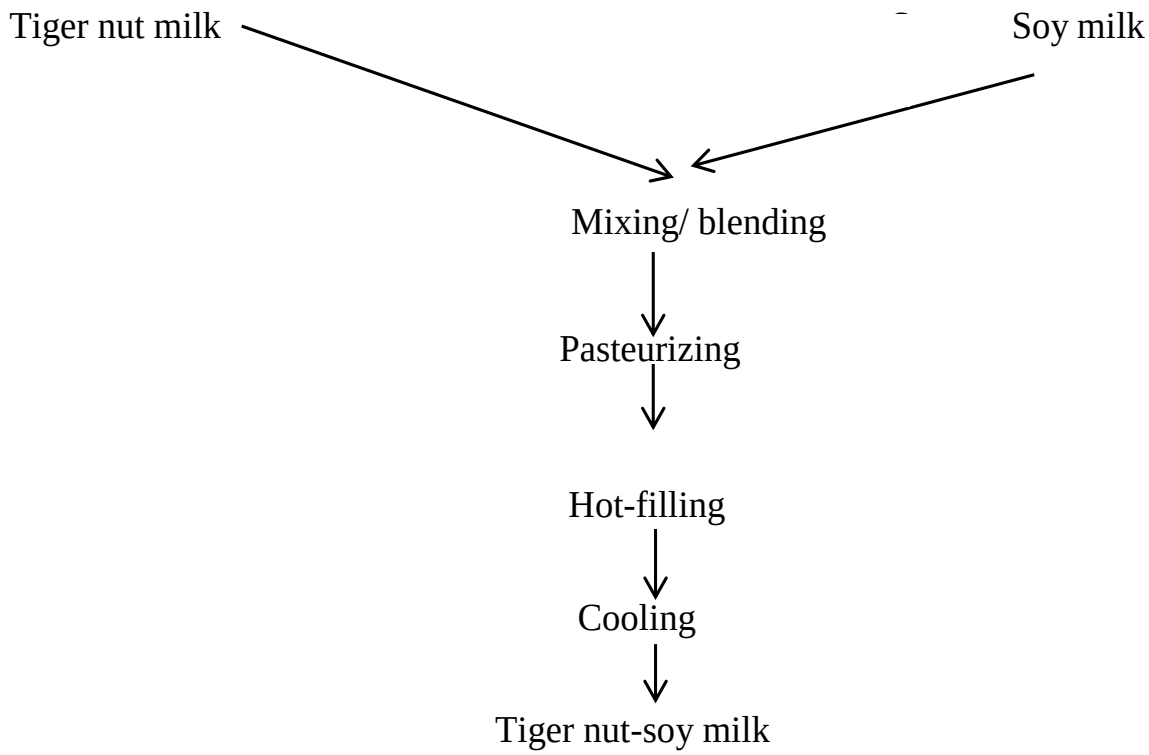

Figure 5. The flow chart for tigernut-soy milk drink production.

0.0261<0.05; the interaction between soy milk and water (BC) with p-value of 0.0189<0.05 as well as the intractions among the three componets (tigernut milk, soy milk and water) (ABC) with a p-value of 0.0056 which is less than 0.05. The other factors of the model were not statistically significant.

The value of the determination coefficient R^2 (0.4499) as shown in Table 6 indicates that the sample variation of 44.99% is attributed to independent variables and 55.01% of the total variations is not explained by the model. The value of the Coefficient of Variation (CV%) (14.95) gives the precision and reliability of the experiment carried out where a lower value of CV indicates a better precision and reliability of the experiments carried out. Table 7 shows regression coefficients of protein.

The regression analysis of the data on Table 7 produced the following coded equation:

$$Y2: Protein\ Content = +3.88\mathrm{A} + 4.03\mathrm{B} + 0.56\mathrm{C} + 4.97\mathrm{AB} + 11.15\mathrm{AC} + 11.64\mathrm{BC} - 50.29\mathrm{ABC} \quad (2)$$

The model equation (Equation 2) shows that all the positive coefficient terms such as A, B, AB, AC and BC indicate synergetic or favourable effect on the protein yield, while the negative coeffcient of the model terms ABC indicate an antagonistic effect on the protein yield (Betiku et al*.,* 2014).

The interaction effect of ABC is the general determining factor of protein yield as it has the largest coefficient. The linear effect of A and B, interaction effect of AC and BC are secondary factors of the response.

Table 2. Formulation of Tigernut-soy milk blends.

Formulation	A (%)	B (%)	C (%)	D (°C)	E (Seconds)	F (Minutes)
1	21	21	50	60	5	5
2	37	5	50	80	20	15
3	5	37	50	60	20	15
4	5	37	50	80	5	5
5	5	17	70	80	20	15
6	5	37	50	60	20	5
7	5	37	50	80	20	5
8	16	16	60	60	5	15
9	17	5	70	60	20	15
10	37	5	50	80	5	5
11	27	5	60	80	20	5
12	37	5	50	60	5	15
13	16	16	60	60	5	15
14	5	37	50	60	5	5
15	17	5	70	80	5	15
16	37	5	50	70	12.5	10
17	37	5	50	60	20	5
18	5	17	70	60	5	5
19	17	5	70	60	20	15
20	21	21	50	60	5	5
21	5	37	50	80	5	15
22	5	27	60	70	12.5	10
23	11	11	70	80	20	5
24	37	5	50	60	20	5
25	37	5	50	80	5	5
26	17	5	70	75	8.75	7.5

Fat

The fat content of the samples ranged from 1.38 to 3.18% (Table 3). The fat value increased with increasing tigernut milk and decreasing soy milk in the blends when water was kept constant (Figure 10).

Thus, higher percentage of tigernut milk in tigernut-soy milk would increase the fat content more than having a larger portion of soy milk in the blend as seen in Figures 11 and 12. Furthermore, Figures 11 and 12 showed that fat content of the various formulations increased with increase in pasteurization temperature.

The analysis of variance (ANOVA) for the response surface combined linear x linear model of fat content of the blends is shown in Table 8. The model expression developed that relates the fat yield and the six reaction parameters (A, B, C, D, E, F) is considered suitable because its p-value of 0.0166 is less than 0.05. The model F-value of 3.43 implies the model is significant. The model fit was also checked with the correlation factor R^2, which equals to 72.94%.

The only significant factor from the ANOVA analysis is the interaction between water and pasteurization temprature (CD) with a p-value of 0.0194 which is less than 0.05. The other factors of the model are not statistically significant.

The value of the determination coefficient R^2, (0.7294) indicates that the sample variation of 72.94 % is attributed to independent variables and 27.06 % of the total variations is not explained by the model. The value of the Coefficient of Variation CV % (17.67) gives the precision and reliability of the experiment carried out where a lower value of CV % indicates a better precision and reliability of the experiments carried out. Table 9 shows regression coefficients of fat.

The regression analysis of data on Table 9 produced the following coded equation:

$$Y3: Fat\ Content = +2.91\mathrm{A} + 2.00\mathrm{B} + 1.99\mathrm{C} - 0.19\mathrm{AD} - 2.835(\mathrm{E} - 003)\mathrm{AE} + 0.090\mathrm{AF} - 0.037\mathrm{BD} + 0.28\mathrm{BE} - 0.33\mathrm{BF} + 0.76\mathrm{CD} + 0.49\mathrm{CE} + 0.50\mathrm{CF}$$

The model equation (Equation 3) shows that all the positive coefficient terms such as A, B, C, AF, BE, CD, CE and CF indicate synergetic or favourable effect on the fat yield, while the negative coeffcient of the model terms such as AD, AE, and BD indicate antagonistic effect on the fat yield (Betiku et al*.,* 2014).

Table 3. Mean values of the proximate composition of the tigernut-soy milk blends.

Formulation	Carbohydrate	Moisture	Ash	Protein	Fat
1.	6.52	89.28	0.12	5.25	2.34
2.	3.58	86.39	0.30	3.50	2.77
3.	2.23	90.68	0.48	3.33	1.90
4.	1.24	90.72	0.15	4.73	2.10
5.	1.42	90.94	0.26	4.38	3.17
6.	1.21	91.39	0.55	3.51	3.11
7.	3.32	90.61	0.81	5.16	2.19
8.	1.99	91.16	0.59	3.28	1.64
9.	3.69	90.52	0.58	3.94	2.96
10.	0.54	89.04	1.06	3.50	2.70
11.	6.00	90.33	1.39	5.69	2.02
12.	2.37	85.9	0.54	4.38	3.17
13.	2.53	90.88	0.48	4.38	1.87
14.	0.81	90.52	0.98	3.94	2.01
15.	9.00	92.34	3.50	3.50	3.11
16.	7.19	83.27	0.20	4.41	3.13
17.	0.88	84.85	0.43	4.38	3.14
18.	2.78	93.06	0.06	4.59	1.38
19.	3.10	90.55	0.16	4.38	2.21
20.	2.71	88.76	0.56	5.25	2.33
21.	1.95	91.33	0.80	3.5	1.65
22.	1.20	90.66	0.28	5.47	1.62
23.	12.5	91.09	4.38	4.38	3.12
24.	3.51	84.00	0.33	3.5	3.18
25.	0.38	90.08	0.20	3.33	2.86
26.	2.99	92.43	0.35	5.25	1.56

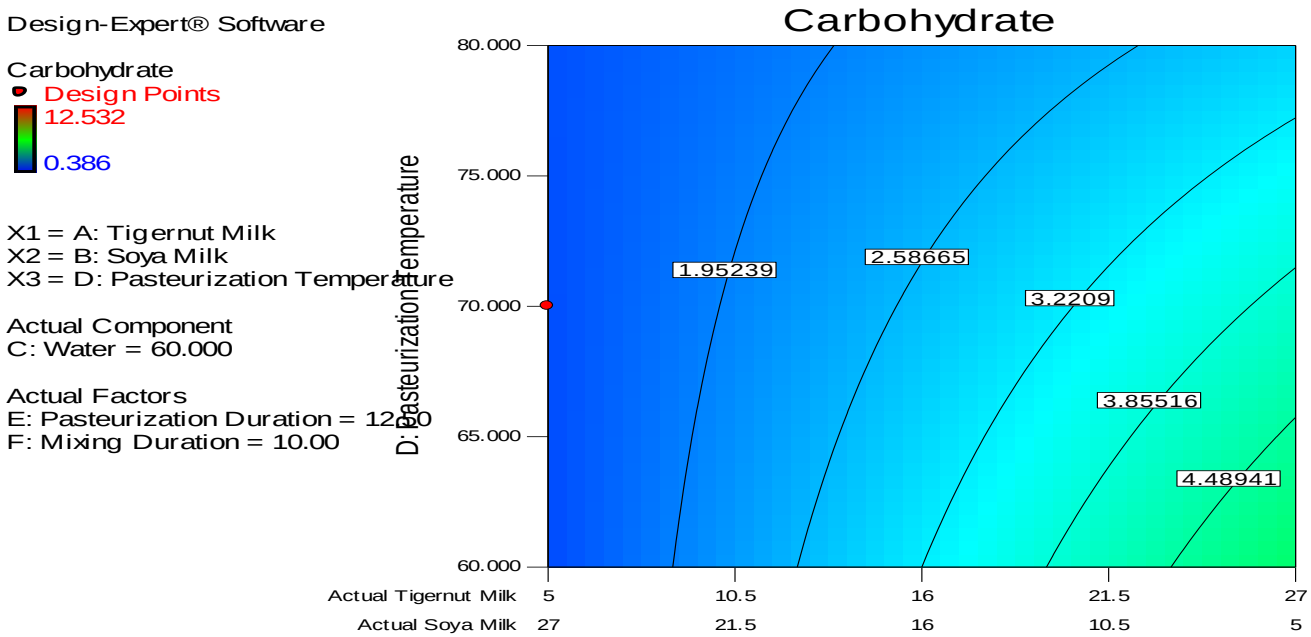

Figure 6. Contour mixed process plots showing the effect of pasteurization temperature on carbohydrate yield at different ratios of tigernut-soy milk blends.

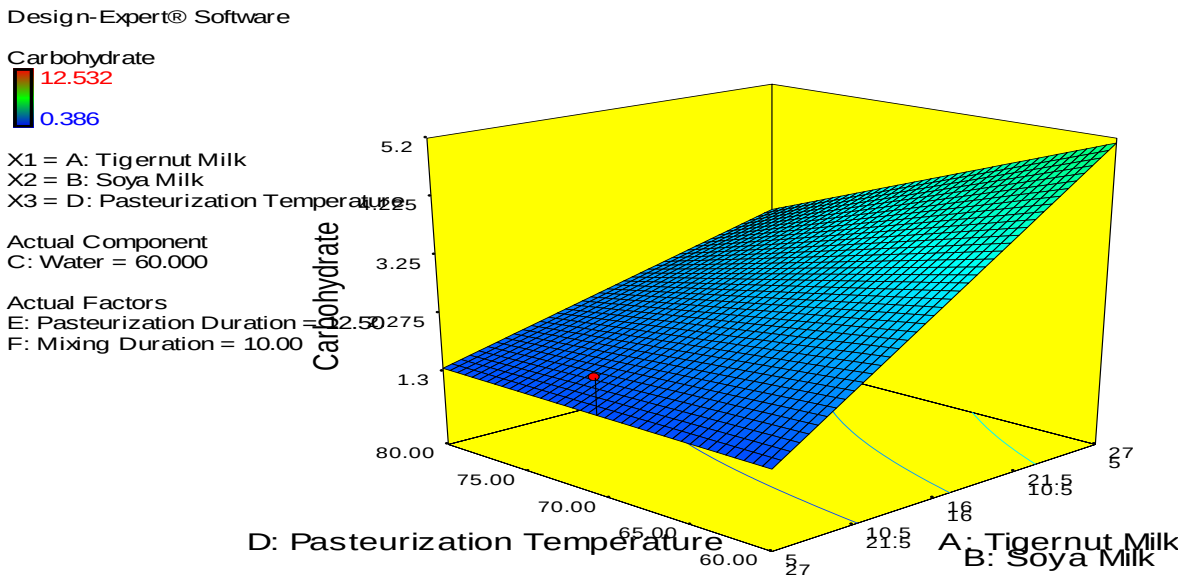

Figure 7. 3-D surface mix-process plots of the various interactions and corresponding carbohydrate yield.

Table 4. ANOVA for combined linear x linear model of the carbohydrate yield.

Source	Sum of Squares	Df	Mean Square	F-value	p-value Prob >F
Model	162.10	11	14.74	5.14	0.0027
Linear Mixture	115.31	2	57.65	20.12	<0.0001
AD	16.73	1	16.73	5.84	0.0299
AE	15.17	1	15.17	5.29	0.0373
AF	0.17	1	0.17	0.059	0.8118
BD	6.207E-003	1	6.207E-003	2.166E-003	0.9635
BE	0.015	1	0.015	5.171E-003	0.9437
BF	3.50	1	3.50	1.22	0.2879
CD	1.810E-004	1	1.810E-004	6.317E-005	0.9938
CE	0.62	1	0.62	0.22	0.6485
CF	0.100	1	0.100	0.035	0.8548
Residual	40.11	14	2.87		
Lack of Fit	25.07	9	2.79	0.93	0.5682
Pure Error	15.05	5	3.01		
Cor Total	202.22	25			

Standard Deviation	1.69
Mean	3.30
C.V. %	51.29
PRESS	129.67
R-Squared	0.8016
Adj R-Squared	0.6458
Pred R-Squared	0.3588
Adeq Precision	8.172

The linear effect of A and B are the general determining factors of fat yield as they have the larger coefficients. The linear effect of C and the interaction effect of CD are secondary factors of the response.

Table 5. Regression coefficients estimates of the carbohydrate yield.

Coefficient component	Estimate	df	Standard error	95% CI Low	95% CI High
A-Tigernut Milk	6.45	1	0.64	5.07	7.83
B-Soya Milk	2.55	1	0.68	1.10	4.00
C-Water	-1.33	1	1.08	-3.64	0.98
AD	-1.60	1	0.66	-3.02	-0.18
AE	1.53	1	0.66	0.10	2.95
AF	0.17	1	0.70	-1.33	1.67
BD	0.032	1	0.69	-1.45	1.51
BE	0.050	1	0.69	-1.43	1.53
BF	0.76	1	0.69	-0.72	2.24
CD	-9.017E-003	1	1.13	-2.44	2.42
CE	-0.54	1	1.16	-3.02	1.94
CF	0.22	1	1.19	-2.33	2.77

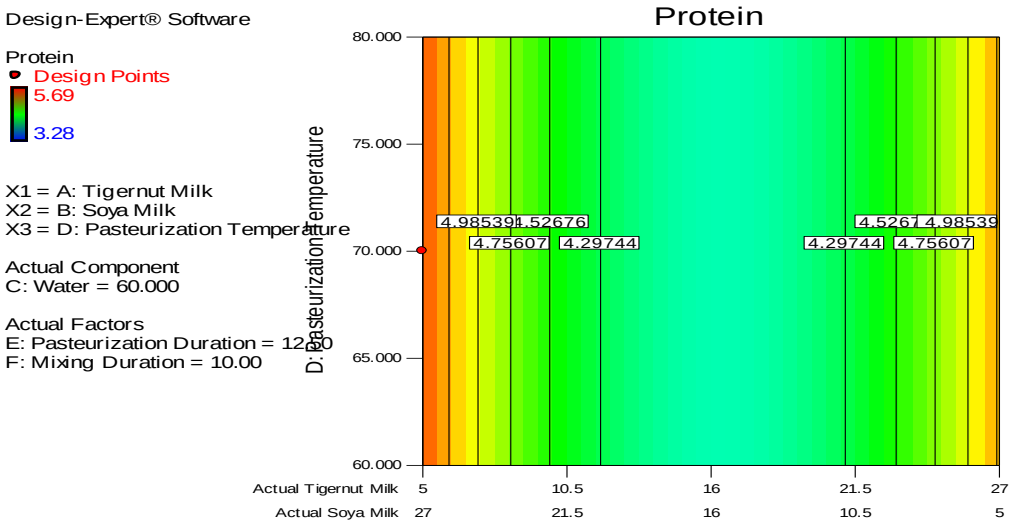

Figure 8. Contour mix-process plots showing the protein yield in relations to the effect of the process parameters.

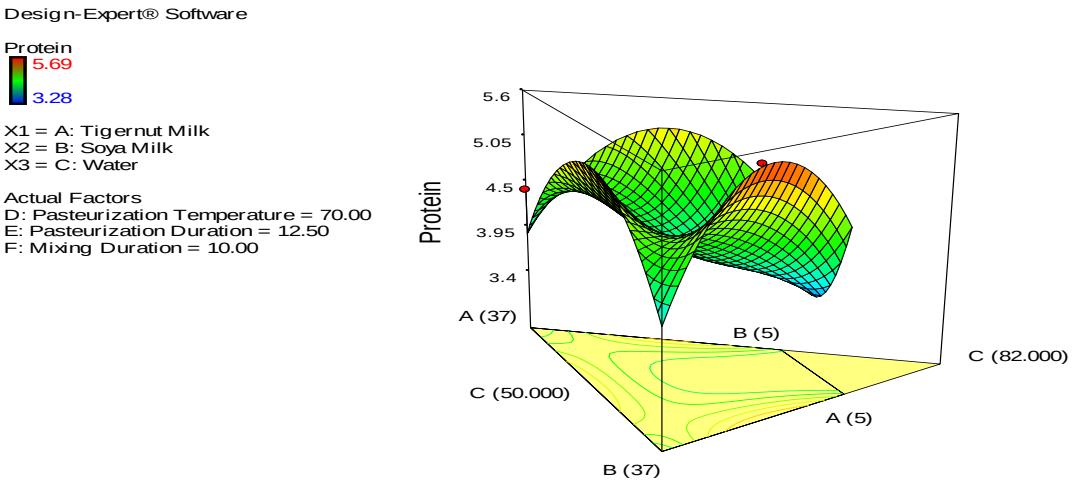

Figure 9. 3-D Surface plots showing protein yield with respect to the effect of the constituents.

Table 6. ANOVA for combined special cubic x mean model of the protein yield.

Source	Sum of Squares	df	Mean Square	F-Value	p-value Prob> F
Model	6.32	6	1.05	2.59	0.0526
Linear Mixture	0.57	2	0.29	0.71	0.5059
AB	2.69	1	2.69	6.63	0.0186
AC	2.37	1	2.37	5.82	0.0261
BC	2.68	1	2.68	6.58	0.0189
ABC	3.96	1	3.96	9.75	0.0056
Residual	7.72	19	0.41		
Lack of Fit	6.62	14	0.47	2.14	0.2052
Pure Error	1.10	5	0.22		
Cor Total	14.04	25			
Standard Deviation			0.64		
Mean			4.27		
C.V. %			14.95		
PRESS			14.04		
R-Squared			0.4499		
Adj R-Squared			0.2762		
Pred R-Squared			0.0004		
Adeq Precision			5.656		

Table 7. Regression coefficients estimates of the protein yield.

Component	Coefficient estimate	Df	Standard Error	95% CI Low	95% CI High
A-Tigernut Milk	3.88	1	0.24	3.37	4.38
B-Soya Milk	4.03	1	0.26	3.48	4.57
C-Water	0.56	1	1.77	-3.14	4.26
AB	4.97	1	1.93	0.93	9.01
AC	11.15	1	4.62	1.48	20.82
BC	11.64	1	4.54	2.14	21.14
ABC	-50.29	1	16.11	-84.00	-16.58

Furthermore, the tigernut milk concentration (factor A) had the highest coefficient among the three independent variables. This implies that the yield of fat from tigernut-soy milk blends relies greatly on this factor as may be clearly visualised in Figure 13.

Moisture content

The moisture content value of the samples ranged from 83.27 to 93.06% (Table 3). This high range of moisture content reveals that the product is highly refreshing, hence a good source of healthy natural refreshment which should be preferred to chemical (synthetic) beverages. The moisture level increased with increasing soy milk and decreasing tigernut milk in the pasteurized blends when water was kept constant (Figure 14).

Figure 15 further buttresses the point. It shows that the higher the percentage of soy milk in tigernut-soy milk blend compared to tigernut milk, the greater the moisture content of the blend.

Furthermore, the range of moisture content of the various formulations after pasteurization shows that the moisture content generally increased with increase in pasteurization temperature (Figure 16).

The analysis of variance (ANOVA) for the response surface combined linear x linear model model of moisture content is shown in Table 10. The model expression developed that relates the moisture yield and the six reaction parameters (A, B, C, D, E, F) was considered suitable because the p-value of 0.0001 is far less than 0.05. The model F-value of 10.67 implies that the model is significant. The model fit was also checked with the correlation factor R^2, which equals to 89.34%.

The significant factors from the ANOVA analysis are the interactions between tigernut milk and pasteurization

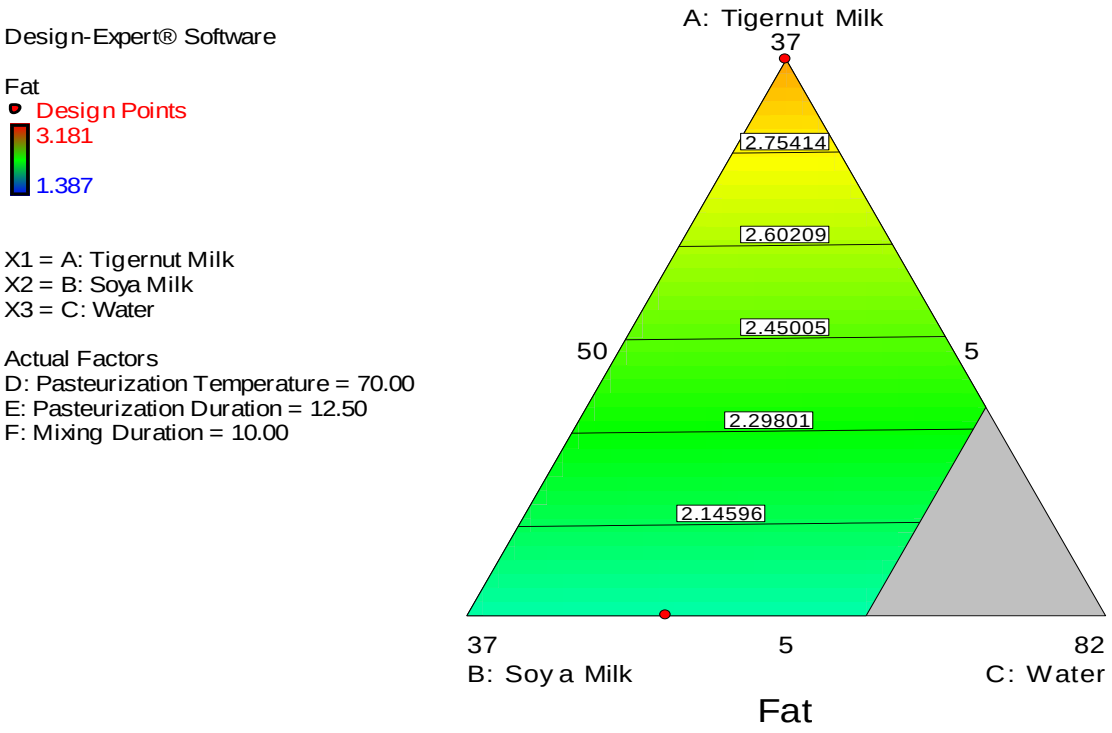

Figure 10. Contour plot showing fat yield as determind by the process constituents.

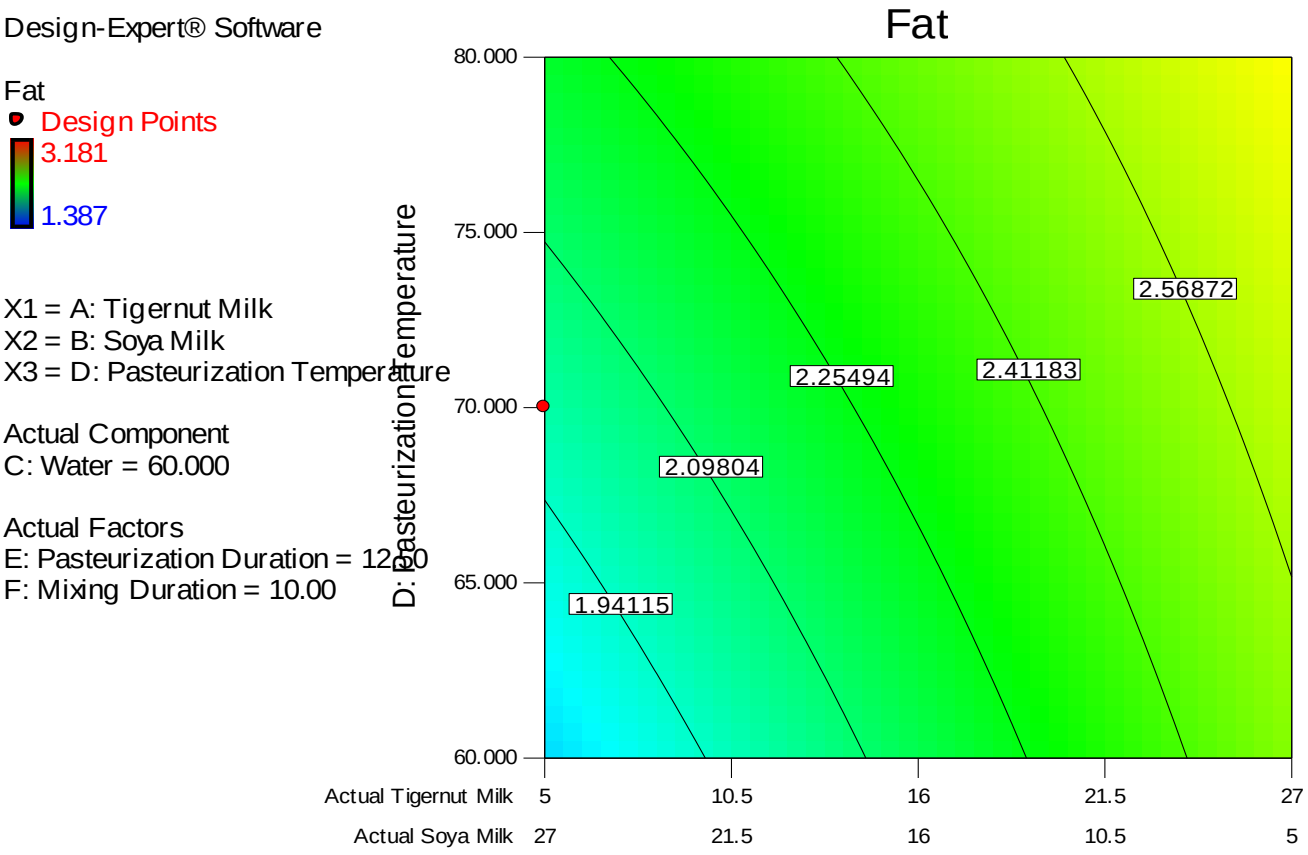

Figure 11. Contour mix-process plots showing the effect of pasteurization temperature on fat yield through the milk blends.

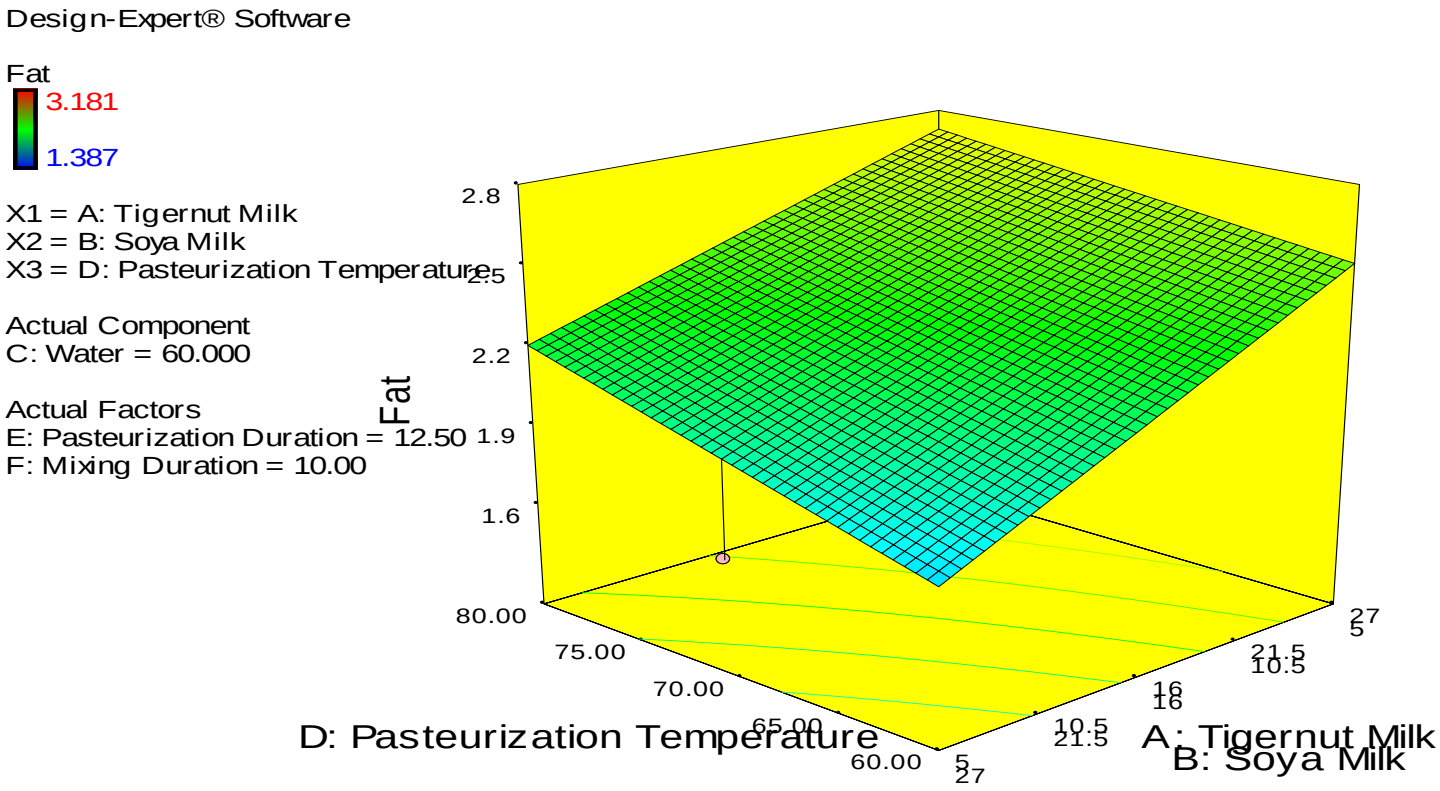

Figure 12. 3-D surface mix-process plots showing the effect of pasteurization temperature on the yield of fat with respect to the milk composition.

temperature (AD) with a p-value of 0.0045 which is less than 0.05 as well as the interaction of tigernut and pasteurization duration (AE) with p-value of 0.0308 which is also less than 0.05. The other factors of the model had no statistically significant effect.

Furthermore, the value of the determination coefficient, R^2 (0.8934) indicates that the sample variation of 89.34% is attributed to independent variables and 10.66% of the total variations is not explained by the model. The value of the Coefficient of Variation, CV % (1.26) gives the precision and reliability of the experiment carried out where a lower value of CV % indicates a better precision and reliability of the experiments carried out. Table 11 shows the regression coefficients of moisture content.

The regression analysis from Table 11 produced the following coded equation:

$$Y4: Moisture\ Content = +86.33\mathrm{A} + 90.73\mathrm{B} + 94.27\mathrm{C} + 1.50\mathrm{AD} - 1.07\mathrm{AE} - 0.63\mathrm{AF} - 0.14\mathrm{BD} - 0.14\mathrm{BE} + 0.023\mathrm{BF} - 0.82\mathrm{CD} - 0.76\mathrm{CE} + 0.22\mathrm{CF} \quad (4)$$

The model equation (Equation 4) shows that all the positive coefficient terms such as A, B, C, AD, BF, and CF indicate synergetic or favourable effect on the moisture yield, while the negative coeffcient of the model terms such as AE, AF, BD, BE, CD and CE indicate antagonistic effect on the moisture yield (Betiku et al., 2014).

Ash content

The ash content, which is the approximate total mineral or inorganic matter of the samples ranged from 0.069 to 4.38% (Table 3).

From the model graph (Figure 17, contour mix-process plot), it may be deduced that the ash content initially reduced drastically, then began to increase as the pasteurization temperature increased.

Statistically, Tables 12 and 13 as well as the contour plots (Figure 18) show that the various components of the blends had no influence whatsoever on whether the ash value increased or decreased.

The 3 D Surface Mix-Process Plot (Figure 19) also revealed clearly that the ash value remained unaltered by the various proportions of tigernut milk and soy milk in the tigernut-Soy milk blends, but it increased with increase in pasteurization temperature.

The analysis of the variance (ANOVA) for the response surface combined mean x 2FI model of the ash yield is shown in Table 12. The model expression developed that relates the ash yield and the six reaction parameters

Table 8. ANOVA for combined linear x linear model of the fat yield.

Source	Sum of Squares	df	Mean Square	F-value	p-value Prob > F
Model	7.00	11	0.64	3.43	0.0166
Linear Mixture	2.60	2	1.30	7.01	0.0078
AD	0.24	1	0.24	1.30	0.2728
AE	5.236E-005	1	5.236E-005	2.823E-004	0.9868
AF	0.047	1	0.047	0.25	0.6223
BD	8.067E-003	1	8.067E-003	0.043	0.8378
BE	0.47	1	0.47	2.56	0.1322
BF	0.68	1	0.68	3.64	0.0770
CD	1.29	1	1.29	6.97	0.0194
CE	0.51	1	0.51	2.74	0.1204
CF	0.51	1	0.51	2.73	0.1204
Residual	2.60	14	0.19		
Lack of Fit	2.27	9	0.25	3.92	0.0735
Pure Error	0.32	5	0.065		
Cor Total	9.60	25			
Standard Deviation			0.43		
Mean			2.44		
C.V. %			17.67		
PRESS			11.97		
R-Squared			0.7294		
Adj R-Squared			0.5167		
Pred R-Squared			-0.2470		
Adeq Precision			7.711		

Table 9. Regression coefficients estimates of the fat yield.

Component	Coefficient Estimate	Df	Standard Error	95% CI Low	95% CI High
A-Tigernut Milk	2.91	1	0.16	2.56	3.26
B-Soya Milk	2.00	1	0.17	1.63	2.37
C-Water	1.99	1	0.27	1.40	2.58
AD	-0.19	1	0.17	-0.55	0.17
AE	-2.835E-003	1	0.17	-0.36	0.36
AF	0.090	1	0.18	-0.29	0.47
BD	-0.037	1	0.18	-0.41	0.34
BE	0.28	1	0.18	-0.096	0.66
BF	-0.33	1	0.18	-0.71	0.041
CD	0.76	1	0.29	0.14	1.38
CE	0.49	1	0.29	-0.14	1.12
CF	0.50	1	0.30	-0.15	1.15

considered (A, B, C, D, E, F) may not be considered suitable because its p-value of 0.2687 is greater than 0.05. The model F-value of 1.39 further implies that the model may not be suitable. The model fit was also checked with the correlation factor R^2, which equals 30.52%.

It was also observed that all the factors had p-values greater than 0.05, thus they are not considered significant. Therefore, it suffices to say that the entire factors of the model have no statistically significant effect.

The value of the determination coefficient, R^2 (0.3052) indicates that the sample variation of 30.52% is attributed to independent variables and 69.48% of the total variations is not explained by the model. The value of the coefficient of variation, CV % (126.24) gives the precision and reliability of the experiment carried out where a lower

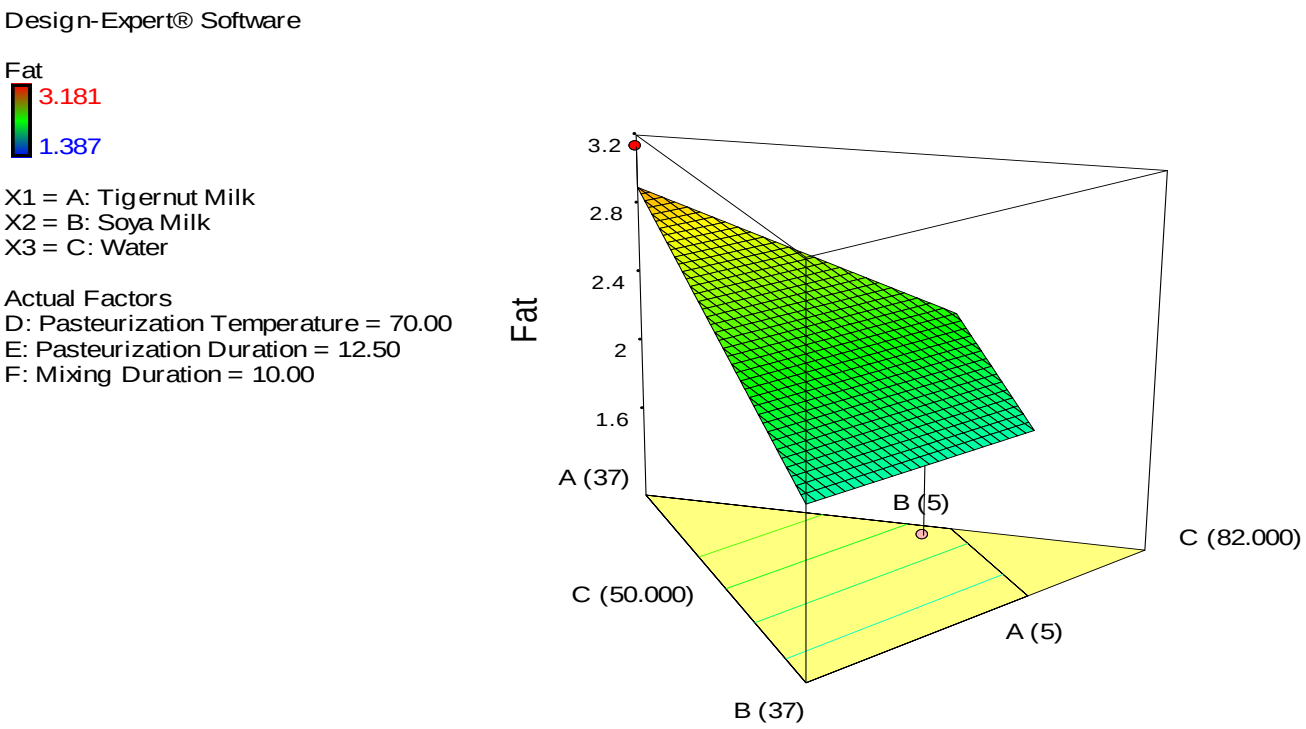

Figure 13. 3-D surface plots showing the relationship between the various constituents and fat yield.

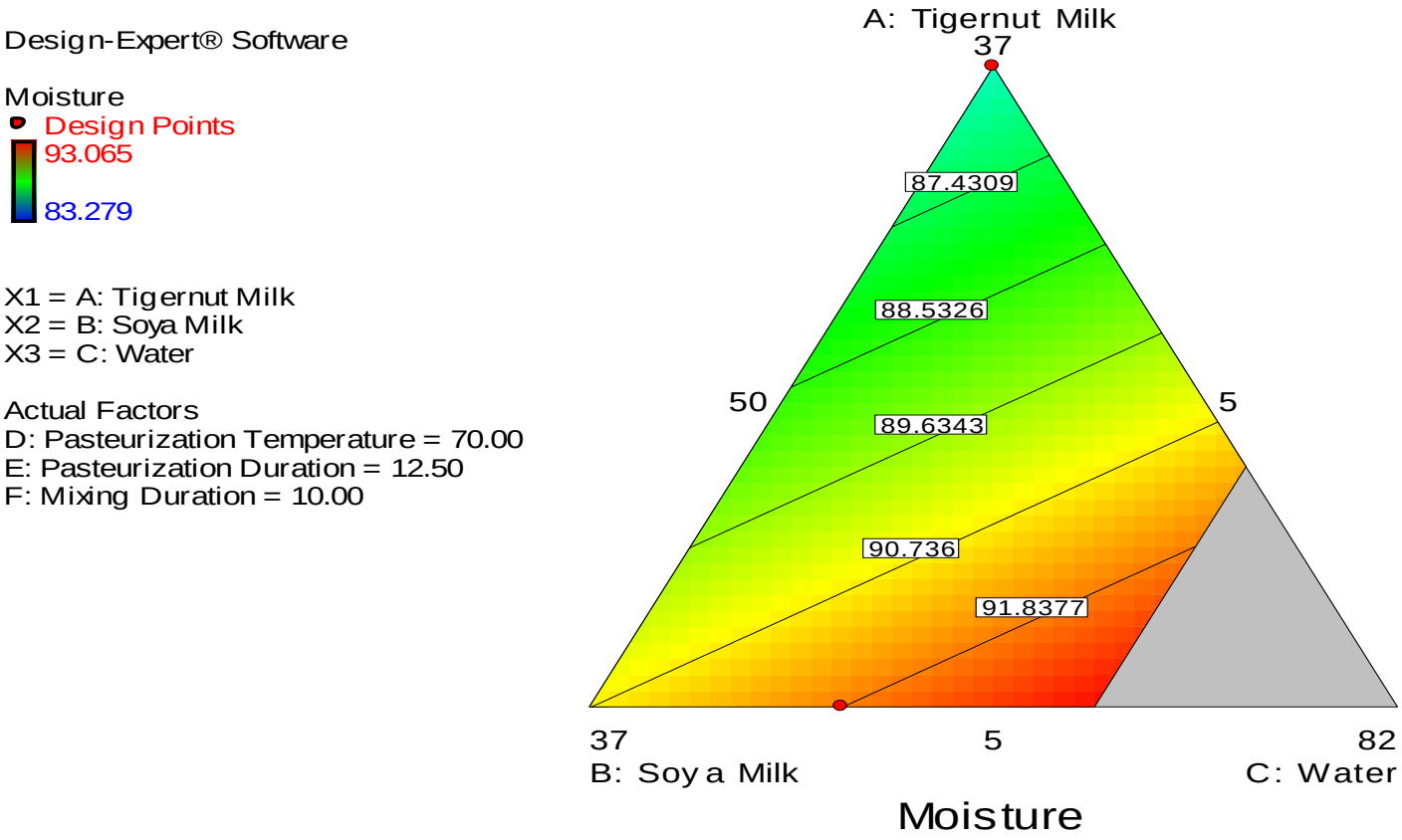

Figure 14. Contour plots showing the relationship between moisture content and the various blend components.

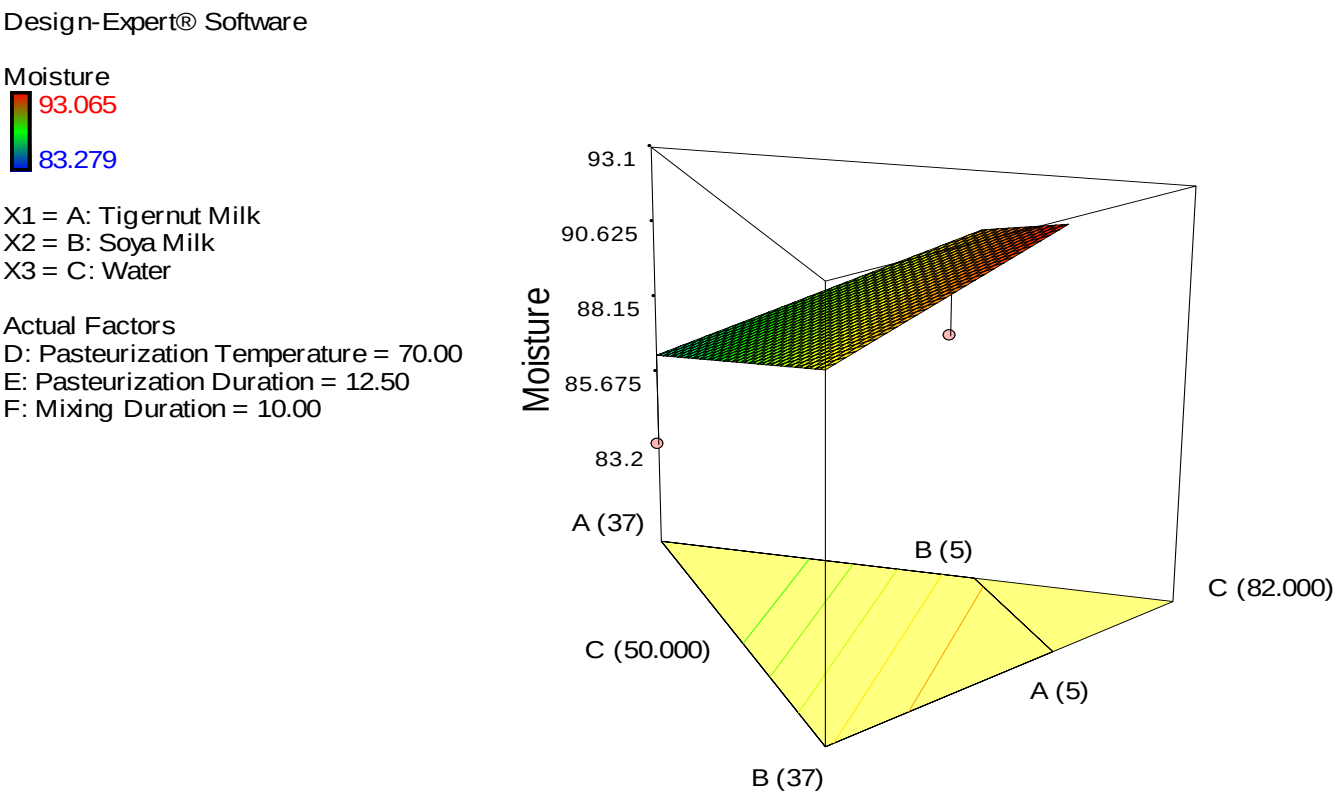

Figure 15. 3-D surface plot showing the relationship between moisture content and the blend components.

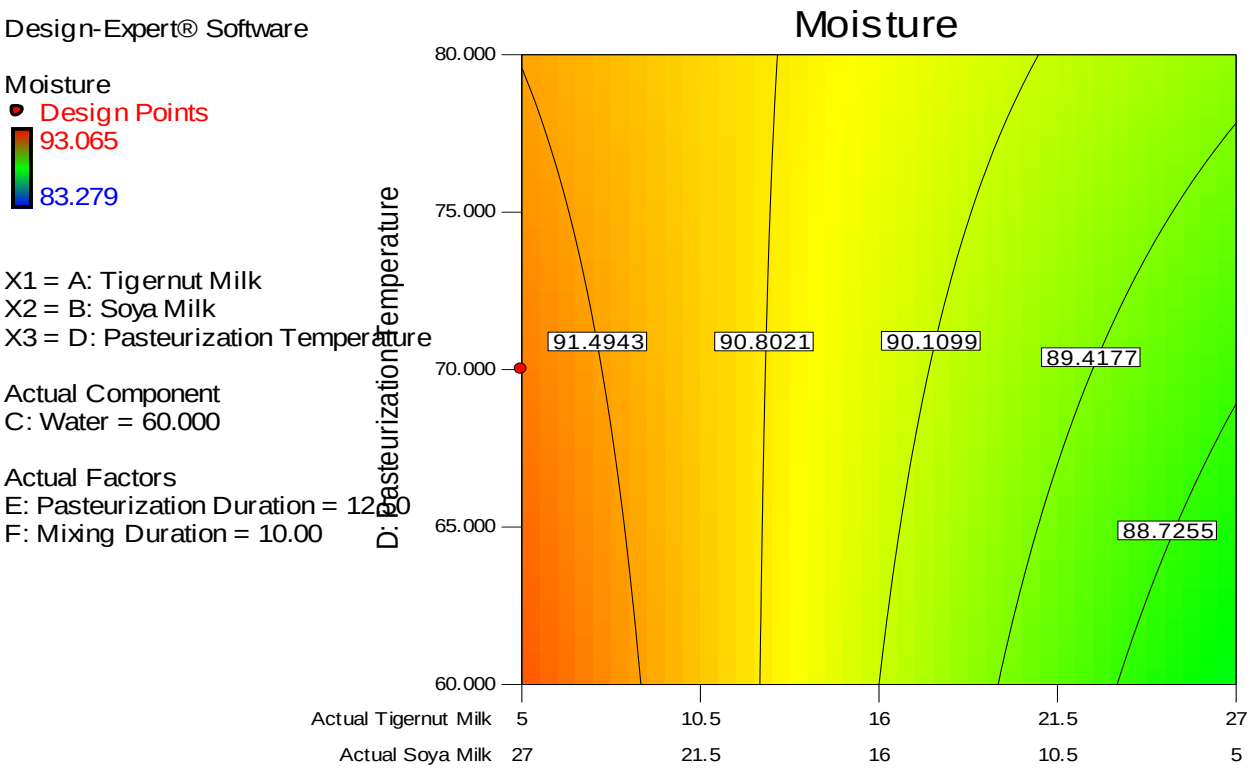

Figure 16. Contour mix-process plots showing the yield of moisture with regards to pasteurization temperature.

Table 10. ANOVA for combined linear x linear model of the moisture yield.

Source	Sum of Squares	Df	Mean Square	F-value	p-value Prob >F
Model	150.80	11	13.71	10.67	0.0001
Linear Mixture	116.04	2	58.02	45.17	0.0001
AD	14.69	1	14.69	11.44	0.0045
AE	7.40	1	7.40	5.76	0.0308
AF	2.31	1	2.31	1.80	0.2016
BD	0.12	1	0.12	0.094	0.7634
BE	0.11	1	0.11	0.086	0.7739
BF	3.112E-003	1	3.112E-003	2.423E-003	0.9614
CD	1.49	1	1.49	1.16	0.2992
CE	1.23	1	1.23	0.96	0.3442
CF	0.096	1	0.096	0.074	0.7889
Residual	17.98	14	1.28		
Lack of Fit	16.91	9	1.88	8.75	0.0139
Pure Error	1.07	5	0.21		
Cor Total	168.78	25			
Standard Deviation			1.13		
Mean			89.65		
C.V. %			1.26		
PRESS			2.67		
R-Squared			0.8934		
AdjR-Squared			0.8097		
Pred R-Squared			0.6879		
Adeq Precision			12.331		

Table 11. Regression coefficients estimates of the moisture yield.

Component	Coefficient Estimate	Df	Standard Error	95% CI Low	95% CI High
A-Tigernut Milk	86.33	1	0.43	85.41	87.25
B-Soya Milk	90.73	1	0.45	89.75	91.70
C-Water	94.27	1	0.72	92.72	95.81
AD	1.50	1	0.44	0.55	2.45
AE	-1.07	1	0.44	-2.02	-0.11
AF	-0.63	1	0.47	-1.63	0.38
BD	-0.14	1	0.46	-1.13	0.85
BE	-0.14	1	0.46	-1.13	0.86
BF	0.023	1	0.46	-0.97	1.01
CD	-0.82	1	0.76	-2.45	0.81
CE	-0.76	1	0.77	-2.42	0.90
CF	0.22	1	0.80	-1.49	1.92

value of CV% indicates a better precision and reliability of the experiments carried out.

The regression analysis from Table 13 produced the following coded equation:

$$Y5: Ash\ Content = +0.38\mathrm{D} + 0.014\mathrm{E} - 0.032\mathrm{F} + 0.068\mathrm{DE} - 0.027\mathrm{DF} - 0.41\mathrm{EF} \quad (5)$$

The model equation (Equation 5) shows that all the positive coefficient terms such as D, E, and DE indicate synergetic or favourable effect on the ash yield, while the negative coeffcients of the model terms such as F, DF and EF indicate an antagonistic effect on the ash yield (Betiku et al*.,* 2014). Also, it shows that the linear effect of D and the interaction effect of EF are the general determining factors of ash yield as they have the larger coefficients.

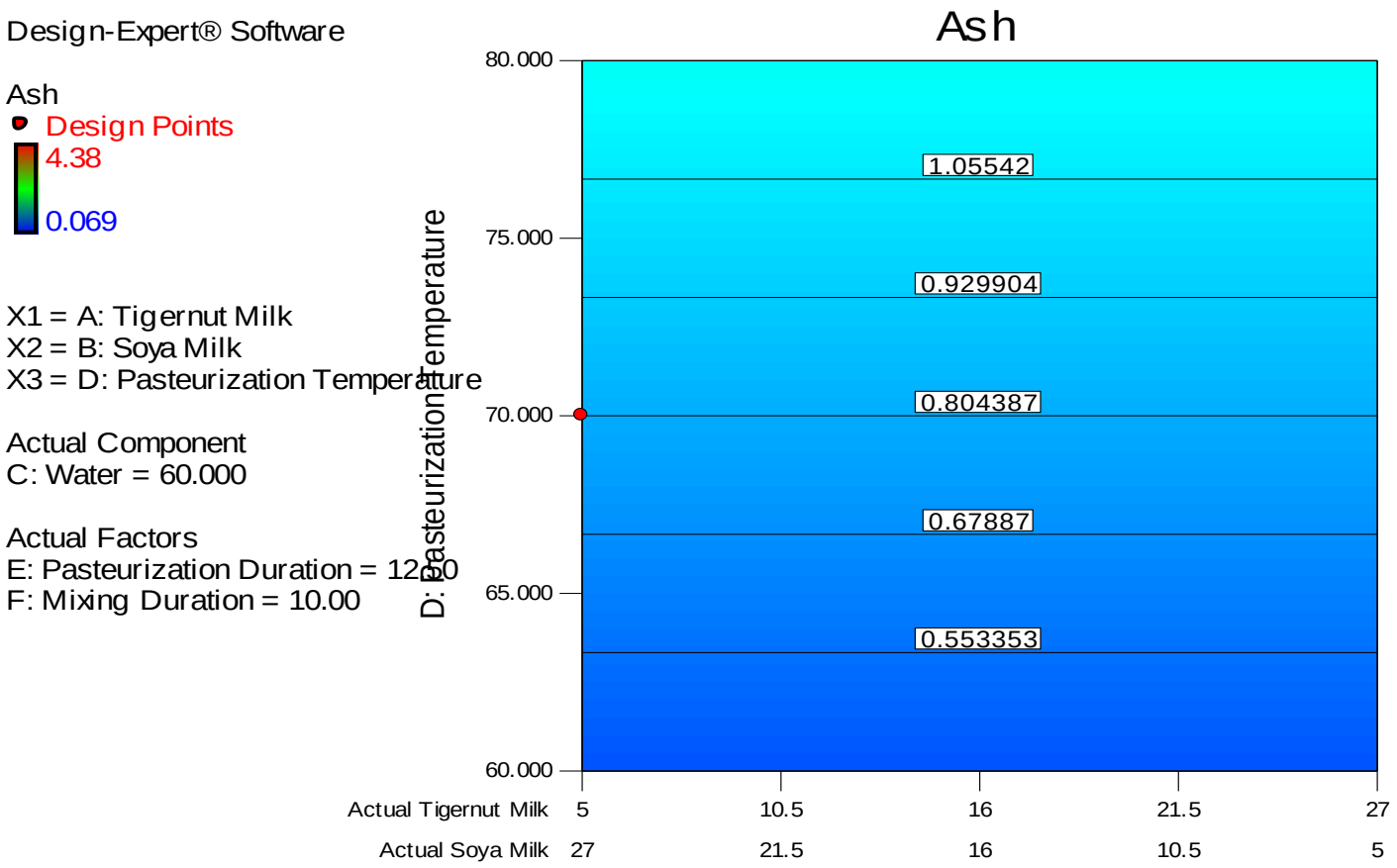

Figure 17. Contour mix-process plots showing fat yield with respect to pasteurization temperatures.

Table 12. ANOVA for combined mean x 2FI model of the ash yield.

Source	Sum of Squares	Df	Mean Square	F-value	p-value Prob > F
Model	7.59	6	1.27	1.39	0.2687
D-Pasteurization Temperature	3.16	1	3.16	3.48	0.0777
E-Pasteurization Duration	4.257E-003	1	4.257E-003	4.681E-00	30.9462
F-Mixing Duration	0.023	1	0.023	0.025	0.8760
DE	0.10	1	0.10	0.11	0.7390
DF	0.016	1	0.016	0.017	0.8967
EF	3.71	1	3.71	4.08	0.0576
Residual	17.28	19	0.91		
Lack of Fit	16.71	14	1.19	10.49	0.0085
Pure Error	0.57	5	0.11		
Cor Total	24.87	25			

Standard Deviation	0.95
Mean	0.76
C.V. %	126.24
PRESS	37.31
R-Squared	0.3052
Adj R-Squared	0.0858
Pred R-Squared	-0.5002
Adeq Precision	3.571

Table 13. Regression coefficients estimates of the ash yield.

Component	Coefficient Estimate	df	Standard Error	95% CI Low	95% CI High
Intercept	0.80	1	0.19	0.40	1.20
D-Pasteurization Temperature	0.38	1	0.20	-0.046	0.80
E-Pasteurization Duration	0.014	1	0.20	-0.41	0.44
F-Mixing Duration	-0.032	1	0.20	-0.46	0.39
DE	0.068	1	0.20	-0.35	0.49
DF	-0.027	1	0.20	-0.45	0.40
EF	-0.41	1	0.20	-0.83	0.015

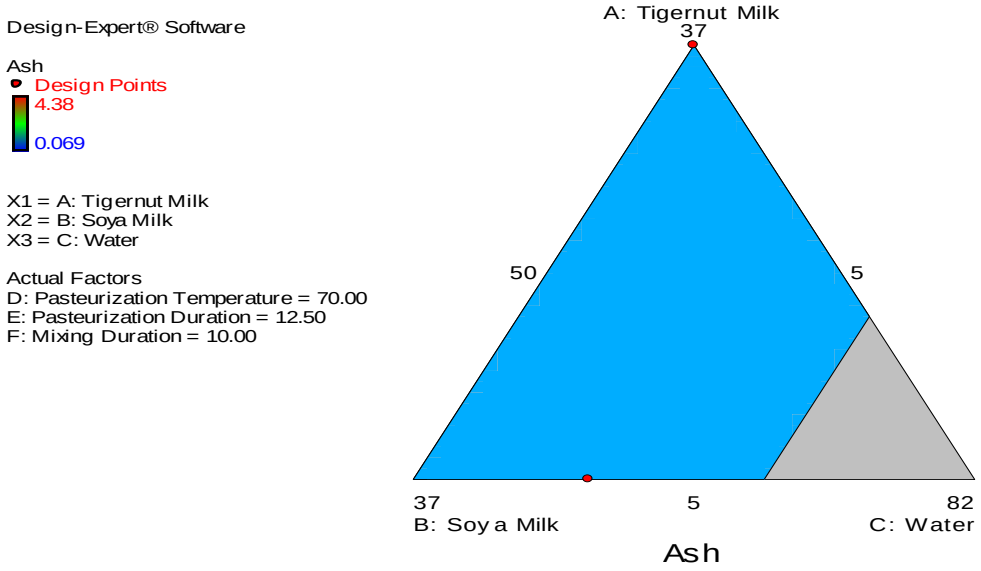

Figure 18. Contour plots showing the relationship between ash content and the various blend components.

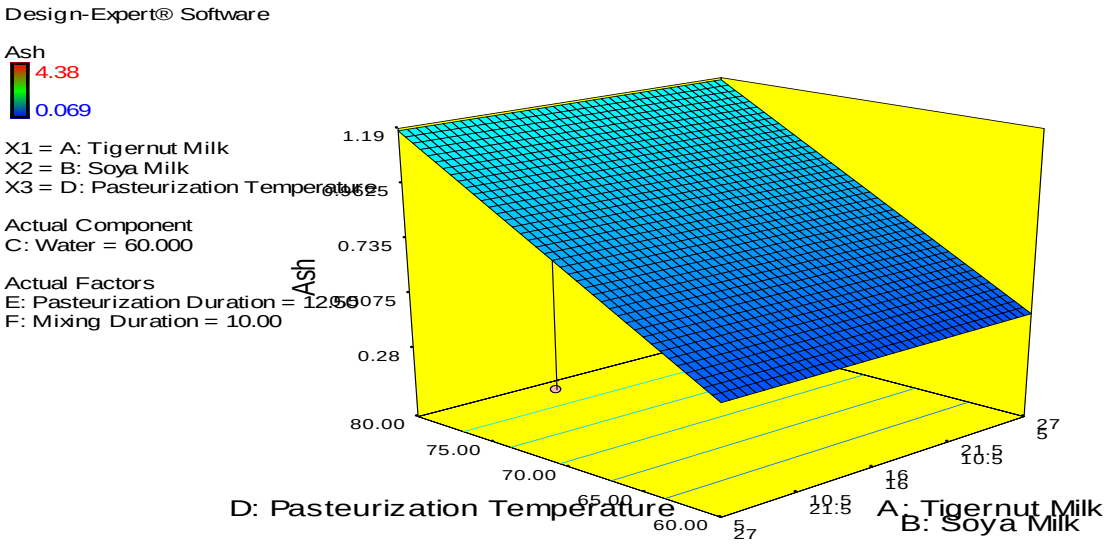

Figure 19. 3-D surface mix-process plots showing the effect of pasteurization temperature on the yield of ash with respect to the milk composition.

Conclusion

This study has shown that pasteurized tigernut-soy milk blend is rich in proximate qualities. When water was kept constant, the higher the percentage of tigernut milk in tigernut-soy milk, the more the carbohydrate and fat content. Moisture content increased with increase in soy milk proportion of the blends while ash values remained unaffected by variations in the tigernut and soy milk ratio. There was no clear-cut direction as to how protein value could be increased or reduced in the tigernut-soy milk blends as the yield of protein fluctuated between increasing and decreasing contents of the blends. Increase in pasteurization temperature reduced the carbohydrate content but favoured increase in fat, ash and moisture content of the blends. There was fluctuation in the protein yield pattern which could be attributable to the destructive effect of heat process involved in pasteurization on the amino acids as well as the tannin-protein complex which had contrast effects.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Supplementary Figures

Figure 1. The LEXUS blender used for mixing the milk and the different milk samples.

Figure 2. The samples set in Water Bath for pasteurization.

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Full Length Research Paper

The nutritional values of two species of sea cucumbers (*Holothuria scabra* and *Holothuria lessoni)* from Madagascar

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Sea cucumbers are fishery products that are not used for consumption by the Malagasy people. In this study, nutritional values of two species of trepangs (air dried sea cucumber) from Madagascar *Holothuria scabra* and *Holothuria lessoni* were determined including moisture, ash, fat, protein and carbonhydrate. The results showed that *H. scabra* and *H. lessoni* had respective water contents of 13 and 14%, the dry matter contents were around 86%. Results on crude protein were around 47% for *H. scabra* and 41% for *H. lessoni*, which constitute almost half of their total composition. Crude ash represented 32% of the dry matter for *H. scabra* and 39% for *H. lessoni.* Lipids and carbohydrates are low (between two and three percent for lipids and about eight percent for carbohydrates). The findings from this study have shown that there are significant differences (p-value <0.005) in nutrient composition in the two species. This low moisture content ensures a long preservation time of the food. These two species are very rich in proteins; this richness in proteins could be an asset in the fight against the malnutrition existing in Madagascar. The high quantity of ash is already sufficient to assume that there are a high quantity and quality of mineral elements. The objective of this study is to determine the nutritional value of the two species for possible presentation to consumers.

Key words: Sea cucumbers, Trepangs, nutritional analysis, *Holothuria scabra*, *Holothuria lesson,* Madagascar.

INTRODUCTION

For many centuries, sea cucumbers have been used for culinary and traditional therapeutic purposes in many Asian countries (Conand, 1994). They have been exploited in Madagascar since 1920, and they rank 6th in terms of economic value compared to other fishery products. The island has a wide variety of these species, but only more than thirty are exploited. Their exploitation is an excellent source of income for fishermen and collectors (between 25000 Ariary and 90000 Ariary per kilogram for dried sea cucumbers, between 500 Ariary and 4000 Ariary per kilogram for fresh ones in 2007 (Eeckhaut et al., 2009) (Ariary is the basic monetary unit of Madagascar). The commercial importance is high, which reached 699 tons in 2012 (MRHP, 2014). All the productions are exported to Asian countries where trepangs (sea cucumber air-dried) are highly appreciated;

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no document mentions its use in Malagasy meals.

Madagascar is a country that presents a wide range of varied pedoclimatic contexts and enjoys a remarkable richness in terms of both its specific biodiversity (flora and fauna) and eco-system, and its soil (MNHN, 2016). The country thus has assets that are a real economic potential. Despite the existence of food resources with high nutritional potentials in the country, more than half of the households in it suffer from chronic or temporary food insecurity (FAO and PAM, 2017). The Malagasy diet is characterized by a predominance of carbohydrate intake (77 to 79%) and a low intake or bioavailability of various important micronutrients and macronutrients, such as proteins (10%) and lipids (11 to 13%), essential fatty acids, iron, vitamins and calcium (Bader et al., 2005). For fighting this problem of nutrition, promoting untapped resources must be a priority in order to increase the availability and variety of foods, especially foods rich in proteins, in minerals and in vitamins. Since Madagascar is an island, coastal environments are likely to provide the population with fishery resources including sea cucumbers.

Sea cucumbers are considered to be excellent nutritional foods, rich in proteins, low in fat, high in amino acids and trace elements (Chen, 2004). Oedjoe (2017) described the nutrient content of fresh sea cucumbers wich inclued protein (21% to 44.07%), fat (1.01% to 1.19%), carbohydrate (0.5% to 2.34%), ash (2.01 to 3.07%) and water (76.03 to 79.43%). Meanwhile Attaran et al. (2017) reported respectively the proximate composition of moisture, ptotein, fat and ash were 93, 4.4, 0.2 and 2% in *H. arenicola.* Hanna et al. (2017) reported that body wall of *H. scabra* (air dried) content around 12.13% of moisture, 55.18% of protein, 27.97% of ash and 1.02% of lipid. However, their nutritional importance remains unrecognized by the majority of the Malagasy population.

The introduction of this food could have some advantages for the Malagasy diet, and hence it seemed useful to study the theme: "the nutritional values of two species of sea cucumbers (*Holothuria scabra* and *Holothuria lessoni)* from Madagascar". The main objective of this study was to determine the nutritional values of two sea cucumber species found in Madagascar, and to measure the acceptance and the appreciation of these products which are new in the Malagasy diet to promote their consumption for they may contribute to the improvement of the dietary diversity. The samples are collected from formed sea cucumber (aquaculture/ holothuriculture) “which is a mode of sea cucumber farming in different types of ponds”. The breeding process is divided into three stages during which the individuals develop at the larval, juvenile and adult stages.

MATERIALS AND METHODS

Sampling

Both species of sea cucumber came from aquaculture or holothuriculture. Batches of processed specimens (dried, also called trepangs) were obtained, then crushed and reduced to flour by a MICROTRON® MB 800 mill in order to be homogenized before.

Determination of moisture content

The moisture composition was determined by calculating the weight loss of the sample while drying in an oven. In a calibrated aluminum capsule; 5 ± 0.001 g of the sample was weighed and dried at 103°C for 12 h in an oven. At the end of the drying process, the capsule containing the sample was cooled in a desiccator under vacuum for about 30 min and then weighed. Moisture content was expressed as a percentage of the weight loss from the original weight (Aubry, 2013).

$$M\ \% = \frac{p.e\ - w}{p.e} \times 100$$

Where: *M %* = moisture percentage, *w* = weight after drying and *p.e* = weight of the test sample in g.

Determination of ash content

The determination of the ash content consists of a gravimetric determination by high temperature calcination. Five grams of sample were weighed then was introduced into a muffle furnace at 550°C for three to five hours, until white, light gray or reddish ash was obtained, apparently free of carbonaceous particles. The capsule containing the ashes was then cooled in a desiccator and weighed. The ash content was expressed as a percentage ratio of the weight of the ash to the oven dry weight (AOAC, 2008).

$$A\ \% = \frac{(w - t) \times 100}{p.e}$$

Where: *A %* = ash percentage, *w* = weight of ash after calcination in g, *t* = weight of the capsule, and *p.e* = weight of the test sample in g

Determination of protein content

The percentage of protein was obtained after the determination of the total organic nitrogen by the Kjeldahl method (AFNOR, 2002). Exactly 0.25 g of sample prepared was weighed. Then 10 milliliter (ml) of concentrated H_2SO_4 and a pinch of catalyst were added to a flask containing the sample. Then everything was placed in a heating system or mineralizer. The reaction was complete only in the absence of undigested material; the solution became clear after 3 h15 min. The tubes were then allowed to cool at room temperature. Their content was quantitatively transferred to Kjeldahl distiller followed by addition of distilled water and 30% (w/v) sodium hydroxide. In a receiving flask, 10 ml of 4% boric acid solution and three drops of Tashiro's reagent color indicator were prepared. The titration was done using 0.1 N HCl directly after the distillation. The mixture of distillate and boric acid was assayed in the presence of Tashiro's reagent until its light green color turned into a persistent pink. Blank titration was carried out in the same way. The percentage of nitrogen content was then calculated by the following formula:

$$N\% = \frac{(\mathrm{V1} - \mathrm{V0}) \times \mathrm{T} \times 14 \times 100}{\mathrm{w} \times 1000}$$

Where: w = weight of the test sample in g, T = title of hydrochloric acid in normality, V_1 = volume of acid used for the test portion in ml,

and V_0 = volume of the acid used for the blank test in ml.

The protein content was calculated using the nitrogen conversion factor of 6.25 [(FAO/OMS, 1973) (Merrill and Watt, 1973)]. The total protein content was obtained by the following formula:

$$Protein\ \% = N\% \times 6.25$$

Determination of lipid content

The extraction and dosage of lipids were carried out by extraction using a mixture of chloroform solvent, methanol and water. One gram of sample and 3 ml of distilled water was homogenized with a mixture of chloroform (10 ml) and methanol (5 ml). It was then stirred for one hour using a magnetic stirrer. The mixture was then vacuum filtered with a sintered glass and a vacuum pump. 4 ml of NaCl were added to the mixture and allowed to settle. NaCl and water separates the homogenate into two layers: The chloroform layer containing all the lipids and the methanol layer containing all the non-lipids. A purified lipid extract was obtained after isolation of the chloroform layer (Folch et al., 1957). If w was the mass of the residue and W the mass of the sample, the lipid level (expressed as a percentage) was given by the following formula:

$$L\ \% = \frac{w}{W} \times 10$$

Where: L % = lipid percentage, w = weight of lipid and W = weight of sample.

Determination of carbohydrate content

The carbohydrate content was deduced from the difference between the total content of the elements and the sum of the protein, lipid, ash and moisture contents.

Percetange carbohydrate = 100 - (% moisture + % ash + % protein + ¨% lipid)

Sensory analysis

For sensory analysis, the hedonic test was performed. The cooked sea cucumber sample was presented in front of the subjects who had to express their opinion regarding the pleasing characters on the rating scale from one to nine. The scale ranged from "extremely unpleasant" to "extremely pleasant", the median expresses that the character was neither pleasant nor unpleasant. A score above five qualifies the appreciation of the product. The test was conducted on 63 naïve consumers, selected from the students residing at the University Campus Ankatso II (36), University Campus Ambolokandrina (13) and 14 people living in the neighborhoods of Ambohipo Antananarivo: 28 women and 35 men, 15 young people who were under 18 years of age (adolescent), 34 young people aged between 18 and 30 years and 14 adults over 30 years.

The scales of quotation proposed by AFNOR (2000) are:

1. This product is extremely unpleasant (bad)
2. This product is very unpleasant (bad)
3. This product is unpleasant (bad)
4. This product is rather (quite) unpleasant (bad)
5. This product is neither unpleasant nor pleasant (neither good nor bad, pleasant or unpleasant)
6. This product is rather (quite) pleasant
7. This product is pleasant (good)
8. This product is very pleasant (good)
9. This product is extremely pleasant (good)

Statistical analysis

All data were processed statistically by the RStudio-1.0.153 software: using the T test and ANOVA.

The T. test was used to find out the difference between the nutrient contents of the two sea cucumber species. This test is a test used to know the difference between two variables by referring to the probability obtained.

The principle is to put a null hypothesis H0 at the beginning which asserts that there is no difference between individuals. After doing the difference test, the probability or p = value is obtained. If it is less than 0.05, H0 is rejected, hence the individuals are different, and otherwise, the samples are similar.

To determine the difference between the means obtained after hedonic test, ANOVA or Analysis of Variance was used. The ANOVA principle follows Fisher's law. The Fisher test consists of comparing the variance between the groups and the variance within the group, the ratio gives the calculated value: F-value which will be compared with the F theoretical obtained by the statistical table (F distribution table) as a function of the DF or degree of freedom and of risk α = 5%.

1. If F-value ≥ F theoretical ((t-1) and (N-t); α) H0 is rejected, at least one of the means is different
2. If Fvalue = F theoretical ((t-1) and (N-t); α) H0 is correct the means are equal

The probability obtained also makes it possible to assert the difference between the means:

1. If p-value < 5%, H0 is rejected, at least one of the means is different
2. If p-value > 5%, H0 is correct the means are equal

RESULTS AND DISCUSSION

The results were divided in two parts: The results of physico-chemical analysis and that of sensorial analysis. Figure 1 and Table 1 show the nutrient levels of the two dried sea cucumbers, *H. scabra* and *H. lessoni*, while Table 2 shows the differences between the nutrient levels of these two species. Tables 3 to 5 present the results of the hedonic test performed on 63 naïve subjects and the analysis of variance of the notes.

Both species of sandfish had low water content: 13.80 ± 1.45 g per 100 g for the *H. scabra* sample and 13.47 ± 0.89 g per 100 g for *H. lessoni* sample. It is due to the practice of smoking and drying processes that remove a significant amount of water during their transformation from fresh to trepangs. As a result, the rate of dry matter was high, about 86%. The moisture content of fresh sea cucumber is approximately 76.3 to 79.43 g per 100 g of edible material (Oedjoe, 2017), which means that approximately 62.83 g of water is removed during treatment. The difference between the moisture content of these two species was not significant (p-value > 0.05). This low water content corresponds to the standard requirement for their quality and conservation, 30% is the maximum tolerable level (Baird, 1974). It proves the good

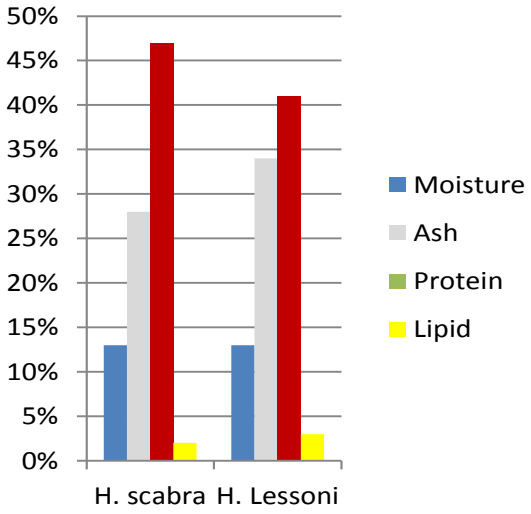

Figure 1. Physicochemical compositions of two species of dried sea cucumbers: *H. scabra* and *H. lesson.*

Table 1. Means and standard deviation (SE) of the physicochemical compositions of two dry sea cucumber species: *H. scabra* and *H. lessoni* (in g per 100 g of sample).

Species	Parameter	Means ± SE
H. scabra	Moisture	13.80 ± 1.45
	Ash	28.18 ± 1.35
	Protein	47.84 ± 2.11
	Lipid	2.070 ± 0.26
	Carbohydrates	8.11
	Dry matter	86.2
H. lessoni	Moisture	13.47 ± 0.89
	Ash	34.51 ± 0.59
	Protein	41.18 ± 2.11
	Lipid	3.020 ± 0.12
	Carbohydrates	7.86
	Dry matter	86.57

Table 2. The difference test between physicochemical compositions of *H. sabra* and *H. lesson.*

Parameter	P-value
Moisture	> 0.05
Ash	< 0.01
Protein	< 0.01
Lipid	< 0.01

quality of these samples, and helps to promote the exportation of trepangs and increase their market value. n fact, the lower their water content, the less the trepangs are perishable, and the longer the preservation is.

Table 3. Mean and standard deviation of sea cucumber ratings

Preferred character	Hedonic value (Mean ± SD)
Odour	5.73 ± 1.49
Taste	5.86 ± 1.71
Global	5.86 ± 1.68

Table 4. Difference of assessment between sex groups: Man and woman (ANOVA Table).

	Df	Sum of square	Mean square	F value	Pr(>F)
Sex	1	28.35	28.350	11.73	0.0011 **
Residual	61	147.36	2.416		

Significant codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1; With Df = 1 and 61, α= 5%, $F_{5\%;1,61}$ =] 3.99; 4.

Table 5. Difference in appreciation between age groups: under 18, between 18 and 30 and over 30 (ANOVA Table).

	Df	Sum square	Mean square	F value	Pr(>F)
Age	2	8.43	4.217	1.513	0.229
Residual	60	167.28	2.788		

With DF = 2 and 60; α = 5%, $F5_{\%;2.60}$ = 4.

The raw ash content provides a rough estimate of the total inorganic matter in the sample. The results obtained in this study showed a high ash content for these two species, about 34 g per 100 g for the *H. lessoni* sample; this was higher than that of *H. scabra*, which was about 28%. The difference was significant for both species, p-value equaled 0.006735 (less than 0.01). The high ash content can reflect a high concentration of minerals, which means that this food is an interesting solution in case of deficiency in these minerals, since it is one of the problems of Malagasy diets. Proteins make up the bulk of the flesh of sea cucumbers or trepangs. In this study, a high protein value was found, and it was almost half the content of the samples, 47.84 ± 2.109 g per 100 g for *H. scabra* sample and 41.18 ± 2.109 g per 100 g for the *H. lessoni* sample. The difference between the protein contents of these two species was significant, p-value equaled 0.01814 (less than 0.05). The results are consistent with data provided by many scientific papers with respect to protein ratios regarding the same or other species. As an example: Ali et al. (2015) found a ratio of about 60% for *H. scabra* and around 53% for other specimens, Hanna et al (2017) found a ratio of 55.18% for body wall of *H. scabra*. The difference in values can be attributed to the influences of several factors, such as the different types of sea cucumber species and the substrates they use during their development. Bouriga et al. (2013) found that fresh sea cucumbers contain between 8 and 14% of protein, and Oedjoe (2017) found between 21 and 44, 07%, which means that the treatments applied to the fresh sea cucumbers during their transformation into trepangs improves protein availability. Compared to protein composition of other foods of fish origin, such as fish and crustaceans whose protein content range between 4 and 26 g per 100 g of sample (Santé Canada, 2008), dried sea cucumbers are assumed to be more nutritious. The high total protein content is a characteristic of sea cucumber species. As a result, it is a food adapted to a diet in regions where the protein-energy deficiency exists, as is the case with Madagascar. They can replace meat and beans as protein rich dishes.

These sandfish species had very low lipid content, of around two percent for *H. scabra* and three percent for *H. lessoni*. Statistically, the composition is different for the two species with p-value equal to 0.01306 (less than 0.05). Sea cucumbers have never been known as a source of lipid, which has been proven by many authors: 100 g of fresh sea cucumber contains around 1.12 g of lipids (Oedjoe, 2017); according to Hanna et al. (2017), dried *H. scabra* contains only about 1.55% of lipids, which complies with a fat free diet. The difference between these two species was significant.

Like any animal flesh, sea cucumbers contain only a tiny portion of carbohydrates. In this study, only eight percent of the flesh consisted of carbohydrates for both species with a difference that was minimal between the two species. These results are comparable to those reported by Berger and Carbonneeau (2014) and by Hanna et al. (2017). Some other authors have found no

trace of carbohydrates in their samples (Ali et al., 2015). During their use as food, a supplementation with a carbohydrate source food is necessary, which means that they are adapted to the Malagasy rice diet. The combination of these two foods is a perfect nutritional supplement.

The means of the hedonic values obtained after the hedonic test were greater than five, which means that this product is appreciated and accepted by the juries even if it is a moderate acceptance. However, for both sex groups (males and females), the assessment is not the same, men value more than women. According to the analysis of variance, F-value = 11.73, which is much higher compared to $F_{5\%;1,61}$ (11.73 >] 3.99; 4 [), and the probability p-value is lower than 0.01 (0.0011 << 0.01), confirming this which states that the means of the grades are different.

In relation to the age of the consumers, the difference of appreciation is not observed as F-value was less than $F_{5\%;2,60}$ (1.51 < 4) and the probability p-value was greater than 0.05 (0.23 > 0.05).

Conclusion

A significant difference was noted for some nutrients between the two species. Sea cucumbers contain a very high protein content of between 40 and 55% and a high ash content of 28 to 34%. On the other hand the lipid content ranges between 2 and 3% and the carbohydrate content between 7 and 8% which are very low. The product was accepted and appreciated by the juries even if it was a moderate appreciation. However, the research remains unfinished. It would be interesting to determine the composition of amino acids and mineral elements. In addition, research on protein valorization would be an advantage for the population.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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